PB-216 956

KINETICS AND MECHANISM IN ACTIVATED SLUDGE PROCESSES

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May 1968

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WP-00325

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WP—00325
PROGRESS REPORT
FWPCA—USDI

Kinetics and Mechanism in Activated Sludge Processes

Period: September 1, 1966, to August 31, 1968

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Report prepared: May, 1968

PROGRESS REPORT

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WP-00325 FWPCA - USDI
(September 1, 1966, to August 31, 1968)

PREFACE

The format for this report is similar to the previous one for Project WP-00325, which covered the period from September 1, 1962, to August 31, 1966. The present report covers the period September 1, 1966, to August 31, 1968. The previous report consisted largely of a compilation of the original manuscripts of sixteen journal articles and papers presented at research conferences concerning work conducted on project WP-00325, and the present report consists largely of ten additional articles on work completed from September 1, 1966, to the present time which was supported wholly or in part by the research grant. The report also covers, in summary fashion, work which is now nearing completion or is in progress and scheduled for completion by August 31, 1969 (in accordance with the research proposal submitted in the summer of 1966 for the period September 1, 1967, to August 31, 1969). Following the detailed summary of work accomplished since the last report and a brief resume of project personnel, abstracts of papers and publications are presented. Finally, the original manuscripts of each paper are presented.

paper are presented. In order to maintain continuity with the previous report, the abstracts and papers are numbered 17 through 26.

DETAILED SUMMARY OF WORK ACCOMPLISHED
(September 1, 1966, to August 31, 1968)
WP-00325 FWPCA - USDI

In general, the detailed summary of work accomplished covers the period from September 1, 1966, to the present time. Work from September 1, 1962, to August 31, 1966, was presented in detail in the previous report and, where appropriate, it is referenced in the present report. The reference numbers appearing in this report (1 through 26) are identified in the bibliography, which is presented in two parts, i.e., 1 through 16 from the report for September 1, 1962, to August 31, 1966, and 17 through 26 for the period of the present report, September 1, 1966, to August 31, 1968). In the interest of continuity, the research topics covered in the detailed summary follow the same order of presentation used in the previous report. Where reference is made to the previous report but not specifically to papers 1 through 16, it is made parenthetically in the text as (WP-00325:01-04).

A. Long-term Kinetic Studies Conducted in Continuous Flow Units

1. Carbon Source as Limiting Nutrient

In the previous report (WP-00325:01-04) the rationale and need for a massive amount of experimentation under "steady state" continuous flow conditions was discussed, and work which had been completely analyzed was reported in paper 16. It was also indicated that work at high substrate levels and studies employing sludge recycle were nearing completion. Additional experimental runs of a spot-checking nature have been made and this phase of the work has now been completed. A detailed report of the findings has been prepared and is presented in paper 17.

These long term studies have comprised one of the primary efforts in the conduct of the project. They have shown that a steady state can be approached for systems employing heterogeneous populations of sewage origin even though such populations are subject at times to rather severe changes in microbial predominance, which can affect the so-called kinetic "constants." It is felt that this finding in itself provides a basis upon which to devise more reliable kinetic formulation for the design and operation of activated sludge processes, since it permits application of more theoretically sound kinetic description of the process and hastens the demise of the essentially empirical design and operational approaches which of necessity are used in present practice and which have not provided reliable operational efficiency. Without reliably high efficiency of secondary treatment processes, the successful function of many of the tertiary treatment processes is endangered.

In general, the work to date has shown that the relation between specific growth rate, μ, and substrate concentration, S (as COD or BOD), is best described by a hyperbolic function similar to the Monod expression rather than by the discontinuous function presently recommended by many engineers (see references 16 and 17). It has also been shown that the value of the kinetic "constants," maximum specific growth rate, μ_m, saturation constant, K_s, and sludge yield, Y, are not true constants for heterogeneous populations but that the range of variation is not so great as to preclude useful engineering prediction of the kinetic behavior of the system with respect to biological solids concentration, effluent quality, and retention time in the aeration tank (16)(17). However, we have concluded that the sludge concentration factor, c, which is defined as the ratio of biological solids concentration in the return sludge to the biological solids concentration in the aeration tank, is not a particularly good parameter for use in the operation of activated sludge systems, and have suggested that a constant concentration of recycle solids (X_R) rather than constant recirculation factor (c) be employed (17). Incorporation of this parameter into the mass balance for the reactor, with respect to biological solids concentration and substrate concentration (as COD), leads to entirely different kinetic equations than those previously employed. We have derived these equations and propose to study their analytical behavior in detail during the terminal year of the research project (September 1, 1968, to August 31, 1969). The mode of operation for activated sludge plants embodied in these kinetic formulas could be more easily facilitated than that employing a constant sludge concentration recycle factor (c) and would lead to less fluctuation in aeration solids concentrations in the aeration tank. Such a mode of operation would allow the system to approximate more closely the true steady state condition.

The new formulations require no changes in the hydraulic rate of sludge recycle, α. The equations we have derived are more complicated expressions than those presented by Herbert, but are more in keeping with the mode of operation and the model needed for activated sludge. We are presently in the process of designing the computational program for assessing the equations under various conditions of sludge recycle flow and recycle sludge concentration, for various ranges of the kinetic constants μ_m, K_s, and Y, and aerator detention times. The derived equations are given below (17). The equation describing the steady state concentration of biological solids, $\bar{x}$, is

$$\bar{x} = \frac{Y\left[S_i - (1 + \alpha)\bar{S}\right] + \alpha X_R}{1 + \alpha}$$

The derivation for the equation for the steady state concentration of substrate (COD or BOD) leads to the quadratic form

$$\bar{S} = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a}$$

where

$$a = \left[\mu_m - (1 + \alpha)D\right]$$

$$b = D\left[S_i - (1 + \alpha)K_s\right] - \frac{\mu_m}{(1 + \alpha)} S_i + \frac{\alpha X_R}{Y}$$

$$c = K_s(D)S_i$$

(Note: all symbols are identified in paper 17).

2. Nitrogen Source as Limiting Nutrient

In the previous proposal requesting research support from September 1, 1967, to August 31, 1969, one of the major subjects for investigation was the study of the kinetic behavior of heterogeneous populations of sewage origin in completely mixed reactors when the nitrogen source (NH_4^+) rather than the carbon source was made the limiting nutrient. Also, it was proposed to determine the value of the kinetic constants, μ_m and K_s, for nitrogen-limited systems. This work has been completed and is reported in detail in papers 18 and 19. Paper 18 deals with the effect of the level of the nitrogen concentration in the feed on substrate removal, biological solids concentration, sludge composition and nitrogen utilization in systems operated at various detention times (reciprocal of dilution rate). It was shown that the dilute-out pattern, as dilution rate or growth rate was increased (detention time decreased) followed, for heterogeneous populations of sewage origin, the same general trend as found for various pure culture studies. It was found that optimum levels of nitrogen supplementation expressed as COD/N ratios could not be based solely on the desired effluent quality with respect to carbon or COD removal since there was a relationship between the amount of nitrogen required, the reactor detention time employed, and the amount of nitrogen utilized by the sludge. Figure 9 of paper 18 is particularly useful, since it shows the relationship between substrate removal and nitrogen content of the sludge for various dilution rates and COD/N ratios. This figure shows how combinations of the system parameters can be used to select the desired effluent quality with respect to carbon source removal and in conjunction with Figure 8 of paper 18, the required level of nitrogen supplementation can be selected with respect to leakage of nitrogen in the plant effluent.

Paper 19 deals with the relationship between the specific growth rate, μ, and the concentration of nitrogen source. In this portion of the research, cells were taken from the completely mixed continuous flow units and used in batch experiments to determine the nature of the above relationships. It was found that a hyperbolic function of the "Monod type" rather than a linear discontinuous function, described the relationship between μ and NH_4^+ concentration; that is to say, the function was similar to the hyperbolic one which could be used to describe the relation between μ and substrate concentration when the carbon source was employed as the limiting nutrient. The range of values observed for the maximum specific growth rate, μ_m, for nitrogen-limited systems was in general accord with that found for heterogeneous populations of sewage origin for carbon source-limited systems. The values for the saturation constant, K_s, were much lower than for carbon-limited systems. This finding is reasonable, since less nitrogen source is needed by the cells. The range of values for μ_m and K_s was established: $\mu_m = 0.4\text{-}0.7\ hr^{-1}$, and $K_{s(nitrogen)} = 1.5$ to 4.0 mg/l.

3. Work at High Sludge Recycle Ratios

In the previous report (WP-00325:01-04) and in the research proposal for the period September 1, 1967, to August 31, 1969, the status of experimentation in completely mixed reactors at various sludge recirculation ratios was discussed, and detailed reports of the work are presented in paper 16 (WP-00325:01-04) and paper 17 of the present report. To date the work has led to the conclusion that "steady state" operation using the parameter "sludge concentration factor," c, is not recommendable from a practical standpoint, since it enhances fluctuation of the biological solids concentration in the reactor (see paper 17 and section A-1 of this detailed summary report). The new kinetic equations for steady state biological solids and substrate concentrations appear to offer a means of steadying such fluctuation. Before they can be used in experimentation, it is desirable that they be studied analytically (mathematically) in order to test their behavior (theoretical dilute-out patterns, etc.) for various values of the kinetic constants (μ_m, K_s, Y) and operational parameters (dilution rates). Since analysis of the equations should be accomplished prior to further laboratory experimentation based on this operational model, further experimentation at various recycle ratios has been delayed. In any event, the results to date indicate that for improved stability, systems should be run at constant concentrations of recycle sludge, X_R, rather than with a constant sludge concentration factor, c.

A considerable amount of experimentation has been accomplished during the past year on systems employing total cell recycle. Work at this particular recycle ratio was stressed because it bears heavily on the so-called "extended aeration" or total oxidation process. This work, which was initially undertaken under this project simply as one of the recycle ratios to be investigated, has developed rapidly and has yielded considerable insight into the total oxidation process. This work appears to merit a greatly expanded experimental effort, and it will become the subject of a separate research effort during the coming year. This separate research project (institutionally supported) will complement the work already accomplished on WP-00325, and will provide for a deeper insight into one of the increasingly used modifications of the activated sludge process.

To date we have found that as the sludge ages in the system due to total retention of solids, the unit activity (expressed as either oxygen utilization ability per gm of sludge or as unit substrate removal capability) has decreased during the period of operation of the unit (over 8 months). However, the total purification capacity of the process has remained high, ± 95% COD removal. It would appear at this stage of the investigation that the sheer numbers of the feeding population make up for the lower feeding activity of individual organisms. Although the process should eventually break down because of increasing cell age, it can be seen from a practical engineering standpoint that if such breakdown does not occur for a fairly long time (i.e., 2-3 years) and if the breakdown time can be predicted, the process holds promise of economic advantage in capital cost of the treatment plant and could enjoy wider application than is presently practiced. It is the purpose of this work to gain insight into this aspect of the total oxidation process under varying but known and highly controlled experimental conditions. Thus far, experimentation has been conducted under long term continuous flow conditions using fairly high waste strength (500 mg/1 and 1000 mg/1 COD) and a reactor detention time of twenty-four hours ($D = 1/24\ hr^{-1}$). In general, the sludge settleability has been rather good (comparable to any other activated sludge process). In the few instances when the sludge has bulked, solids are not lost (a condition which has caused many problems and uncertainty in analyzing results of various reports on the process) because when this condition has occurred, the overflow has been passed through a Sharples centrifuge and the solids are placed back into the aerator. The only solids escaping from the process are the small amounts taken for determination of biological solids concentration and measurement of the biochemical composition of the sludge (less than two per cent of the solids are thus lost). It is felt that complete analysis and conclusions concerning this work should not be attempted until the process either exhibits loss of purification efficiency or has operated for a long enough time without breakdown to support its recommendation for wider application in the pollution control field.

4. Progression from High to Low Dilution Rates Without Cell Recycle

In the previous proposal requesting research support from September 1, 1967, to August 31, 1969, one of the research efforts to be undertaken during this period involved the continuous operation of a completely mixed reactor at various dilution rates beginning with high values of D (low reactor detention times) and proceeding to increasingly lower D values (high reactor detention times). This work was needed because our previous studies had all proceeded from low to high dilution rates (until cell dilute-out occurred). It was felt that this operational procedure provided a selective pressure and that this could have caused (due to a shift in predominance of species in the heterogeneous microbial population) the slightly higher values of μ_m which were observed at high dilution rates. Analysis of the results of the previous experiments did show a trend toward increasing μ_m values as D was increased, but there was considerable scatter of the data, and the trend toward increasing μ_m values was slight (see Figure 9 of paper 17).

However, it was considered important to make these studies in order to verify the validity of the tentative conclusion that the progression of dilution rate (or of detention time), either increasing or decreasing, had little or no effect on the range of μ_m values which would be observed. With the intent of enhancing broader applicability of the data, a sugar alcohol, glycerol, rather than a pure carbohydrate, was selected as carbon source. Also, the aim of the study was extended somewhat in that it was decided to extend the runs to extremely low dilution rates. There was no information in the literature (either basic or applied) which shed any light on the lowest value of D (or specific growth rate μ) which would still allow the population to approach a steady state.

A continuous flow reactor has been in operation for over a year and steady state data have been obtained at increasingly slower growth rates of 1/4, 1/12, 1/24, 1/36, 1/48, 1/72, 1/96, 1/192 hr^{-1}. It is significant to note that a steady state can be approached for dilution rates as low as 1/192 hr^{-1}, i.e., at cell generation or doubling times of 192 hours. This information is extremely important, since it indicates the possibility of employing completely mixed steady state reactor kinetic theory in various slow rate activated sludge processes employing long detention times (e.g., mechanically aerated lagoons). During this extended period of experimentation, samples of the sludge were taken for COD and biological solids determination and for glycerol concentration (periodate method). Also, cells were taken for separate "batch" studies to determine specific growth rate and sludge yield. The work has required such a massive experimental effort that not all of the data has yet been fully analyzed. The experimental portion of this work is now completed, and various check runs have been made. Preliminary analysis of the results indicates that continuous flow operation at very high detention times (or low specific growth rates) does not seriously affect the value of the maximum specific growth rate. However, final conclusions concerning this portion of the project research should not be made until complete and detailed analysis of the results are made. This will be accomplished during the coming year of the project.

5. Mixtures of Various Carbon Sources

In the previous proposal requesting research support from September 1, 1967, to August 31, 1969, one of the planned research efforts which would both provide valuable information on kinetics and mechanism of substrate removal and aid inter-related analysis of results of research projects WP-00075 and WP-00786 (both of which will terminate as of October 31, 1968) was the study under continuous flow operation of multi-component carbon sources. During the past year, batch studies using the following three-component carbon source systems were investigated:

1. Glucose-xylose-sucrose (population acclimated to sucrose)
2. " " " (" " xylose)
3. " " " (" " glucose)
4. Glucose-galactose-sucrose(" " galactose)
5. " " " (" " sucrose)
6. " " " (" " glucose)
7. Glucose-sucrose-glycerol(" " sucrose)
8. " " " (" " glycerol)

For all studies, control systems utilizing each compound as sole source of carbon were also run. All systems were analyzed for total COD, specific carbon source, and biological solids. The batch studies have shown that the order of speed of removal for systems 1, 2, and 3 is glucose>sucrose> xylose, regardless of the "acclimation history" of the microbial population.

The substrate removal response for systems 4, 5, and 6 is much more complicated. In general, glucose was removed first, but the order of removal for galactose and sucrose depended somewhat on the acclimation history of the population and upon the rate of glucose elimination. For systems 7 and 8, the order of removal rate was glucose>sucrose> glycerol, and the mode of removal was dependent upon the acclimation history of the population. For example, with cells acclimated to sucrose, the sucrose and glucose were utilized concurrently (with glucose removed at a faster rate), but utilization of glycerol was completely blocked until all of the sucrose and glucose had been metabolized. These results await more complete analysis, but they have already provided evidence of substrate competition and repression of acclimation to a carbon source due to the presence of another. These substrate combinations will be studied under continuous flow conditions employing various dilution rates (or detention times), since the batch studies have indicated that the rate and the order of removal will depend upon detention time. The results of the continuous flow studies should provide extremely useful information concerning kinetics and mechanism of substrate removal, particularly with respect to minimum aeration tank detention times which can be employed without fostering leakage of carbon source from the reactor (i.e., decreased overall efficiency). The results using these compounds, which are structurally related, can provide guideline information of a conservative nature on minimum detention times for more complicated waste systems in the field, some of which contain a number of structurally unrelated compounds. These studies, when completely analyzed and interpreted in the light of knowledge concerning various whole wastes, may provide insight into more reliable design procedures than are presently employed in the field.

B. Studies on the Exertion of Biochemical Oxygen Demand

In the previous report (WP-00325:01-04), the need for studying exertion of biochemical oxygen demand (O_2 uptake) was delineated and the rationale for the approach taken was discussed. A detailed account of the work on this aspect was presented in papers 4 through 12 of the previous report.

In the course of other work in our laboratories, pertinent to stream reaeration, an apparatus was designed and operated which allowed accurate determination of the reaeration constant K_2 under conditions closely approximating the mode of O_2 transfer in natural receiving streams (Isaacs, W. P., and A. F. Gaudy, Jr., Jour. San. Eng. Div., ASCE, SA2, 319, 1968).

It was reasoned that if K_2 could be accurately determined, then one could add a known amount of organic matter to the simulated stream, determine the DO at various times after addition of the organic matter (appropriately seeded with an acclimated microbial population of sewage origin), and from a knowledge of the "sag" curve and K_2 calculate the O_2 uptake (exertion of BOD) in the stream. The apparatus used was quite large; it provided for nearly 700 l of aerating liquor (see paper 20). Studies were conducted in the simulated receiving stream and in BOD bottles in order to compare BOD exertion under both conditions. It was found that O_2 uptake data obtained in BOD bottles could be used to make a reasonable prediction of the observed minimum or critical DO, provided that the seed concentration in the BOD bottle was identical, or nearly so, to the biological solids concentration in the receiving stream at the time of introducing the organic pollutant and provided that a phasic, rather than one continuous first order, expression was used to describe BOD exertion. The findings are reported in detail in paper 20. Work on BOD exertion using the simulated stream, BOD bottles and a reactor in which turbulence is highly controlled is continuing, using a variety of carbon sources and municipal sewage.

C. Role of Solids Concentration in Determining the Kinetics and Mechanism of Substrate Removal

In the previous report (WP-00325:01-04) the results of investigations on the effect of sludge concentration on the relationship between the rate of substrate removal and initial solids concentration, sludge yield, and the percentage of the chemical oxygen demand exerted at the time of substrate removal were reported in detail (paper 13). Additional studies were run at initial biological solids concentrations in excess of 2000 mg/l, which verified the previous results and extended the knowledge pertaining to immediate uptake of carbon source. This work also verified the biochemical rather than adsorptive mechanism for explaining rapid substrate removal (paper 15). Most importantly, the work provided a sound explanation for linear substrate removal and solids accumulation in systems employing high biological solids concentrations (papers 13 and 15).

Since the previous report, twelve experiments have been run at extremely high initial biological solids concentrations and various substrate concentrations. At biological solids to substrate ratios of one or above, linear kinetics (zero order) were nearly always observed. For many of the experiments, a gas absorption train was set up in order to capture the gases and any possible volatile compounds produced by the organisms during purification of the synthetic waste. In these experiments, glucose was used as the carbon source in the synthetic waste. This compound is not volatile, but in many studies we have observed that metabolic intermediates and/or end products accumulate in the aeration liquor. Special investigation on the causes and the magnitude of production of these products is covered in detail in Section F. In the present work pertaining to effect of high biological solids concentration on kinetics and mechanism of substrate removal, intermediates might be expected to be elaborated by the cells and it appeared likely that some of these products might be volatile to varying degrees and could be stripped from the reactor. Accordingly, the gas train apparatus was employed. This additional investigation seemed justified because, if enough material were stripped, it could affect calculation of materials balances for the systems, and if high amounts of volatile materials were produced and stripped within the immediate atmosphere of a treatment plant, a hazard to plant personnel might ensue. It was found that in many cases intermediate products did accumulate in the mixed liquor and a portion of these were subject to stripping. Compounds such as acetic, propionic, and valeric acids and ethanol were found in the gas train absorbates as well as small quantities of isovaleric, isobutyric, and hexanoic acids. In some of the experiments over 200 mg/l COD was due to stripping and was removed in the gas train absorbate. Results of these twelve experiments are presently undergoing detailed analysis.

D. Studies on Substrate Removal and Regulation of Nitrogen Supply for Nitrogen-deficient Wastes

In the previous report (WP-00325:01-04), the studies leading to proposal of a new modification of the activated sludge process were summarized and a detailed report was presented in paper 14. The work reported as "in progress" concerning the development of this process has now been completed and is reported in papers 21, 22, and 23. The work reported in paper 14 led to proposal of the process for nitrogen-deficient wastes of a carbohydrate nature (e.g., cane sugar wastes and certain pulp and paper wastes). As a result of the work which has just been completed, it is possible to recommend the process for wastes of a noncarbohydrate nature, e.g., acetic acid was used as carbon source (papers 21 and 22). Also, waste components for which degradation proceeds through utilization of inducible enzyme(s) can be successfully treated (paper 21). Extensive laboratory pilot plant studies have shown that the recommended mode of operation can sustain continuous "oxidative" assimilation (continuous removal of the carbon source in the absence of a source of nitrogen) and that the sludge can be "regenerated" by adding nitrogen in an endogenous phase (papers 22 and 23). The process leads to a considerable savings in the cost of the nitrogen supplement (NH_4^+). Using acetic acid, ninety per cent removal of COD was accomplished using a 4-hour detention time and a COD/N ratio of 70/1 (BOD/N = 49/1). This represents a considerable savings in nitrogen compared to the usual BOD/N ratio of 20/1 usually employed. Also, the process can be operated without leakage of supplemental nitrogen in the plant effluent.

In addition to the above work, it is also interesting to note that the process has been tested in a laboratory scale continuous flow pilot plant using a whole waste: cane sugar refinery waste (Gaudy, A. F. Jr., K. C. Goel, and A. J. Freedman, Proceedings 4th International Conference on Water Pollution Control Research, Prague, Czechoslovakia, September, 1968). The process provided excellent treatment efficiency of the whole waste at a COD/N ratio of 60/1. The lower limit of nitrogen supplementation was not explored, and it is possible that even higher COD/N rations can be used.

More laboratory research could be conducted on the process, but it is concluded from the considerable amount of research which has already been conducted at the laboratory scale (papers 14, 21, 22, and 23) that the process is now ready for a larger scale pilot plant (or possibly full scale) effort using various nitrogen-deficient industrial wastes. This part of the project WP-00325 has been particularly gratifying to the principal investigator, since the basic engineering research conducted on the project for the purpose of delineating the kinetics and mechanism of activated sludge processes has led to the conception and testing of a new modification of the process. Such a development is in keeping with the original aims of the project and, in the view of the principal investigator, provides justification for basic engineering research in the water pollution control field and is in keeping with the technical philosophy of engineering research, i.e., the proposed process modification for nitrogen-deficient wastes offers an economic advantage (savings in costs of nitrogen supplementation) and complete assurance that secondary plant effluent will not contribute to stream enrichment problems due to nitrogen leakage.

E. Studies on the Effect of Agitation and Oxygen Tension on the Mechanism and Kinetics of Substrate Removal

In the previous report (WP-00325:01-04) the rationale for making studies on the effect of agitation and oxygen tension on the mechanism and kinetics of substrate removal was given and the preliminary studies then in progress were described. Since the last report, extensive experimentation has been conducted in a continuous flow reactor under various controlled degrees of mechanical agitation and under various controlled oxygen tensions in order to gain an insight into the correlated or independent functions of these two parameters essential to the functioning of activated sludge processes. This work has now been completed, and major portions of it are reported in detail in papers 24 and 25. Paper 24 deals primarily with the effect of agitation or mixing energy. The parameter used to determine the degree of mixing energy put into the system was the velocity gradient, G. As G was increased, there was a decrease in the steady state biological solids concentration and an increase in oxygen uptake, i.e., an increasing portion of the carbon was used for respiration rather than for sludge synthesis as G was increased (paper 24). The O_2 uptake was measured continuously by passing the effluent air stream from the reactor through an O_2 analyzer. In other studies (paper 25), the velocity gradient was held constant and the oxygen content of the aerating gas was varied. Operation of the continuous flow reactor under these conditions led to steady state DO concentrations between 1.4 and 7.1 mg/l. The DO concentration in this range had no effect on O_2 uptake or biological solids concentration. One of the primary conclusions to be drawn from the work is that DO concentration of itself exerts no effect on O_2 uptake or sludge yield. Also, since the degree of agitation did affect O_2 uptake and sludge yield, it is concluded that it exerts an effect independent of oxygen tension.

F. Investigations into the Release of Metabolic Intermediates during Substrate Removal

In the previous report (WP-00325:01-04) preliminary experiments undertaken on conditions fostering the release and accumulation in the medium of metabolic intermediates and/or end products produced by the sludge during the metabolism of the initial exogenous carbon source were discussed. The importance of undertaking such experimentation and the rationale for its inclusion in the research on project WP-00325 were delineated. Also, in the research proposal for the period September 1, 1967, to August 31, 1969, the proposed work on the effect of (a) the carbon source, (b) the amount of nitrogen source, and (c) the dissolved oxygen concentration, on the release and accumulation of metabolic intermediates and/or end products was described.

During the past year this work has comprised a major experimental effort. Forty-four long term studies have been conducted both under growth (no nitrogen limitation) and under nonproliferating conditions using a variety of compounds as carbon source. In addition, the effect of the biological solids concentration on the release and accumulation of metabolic intermediates and/or end products has also been investigated. Initial exogenous carbon sources which have been used include acetic acid, glucose, sucrose, propionic acid, glycerol, and sorbitol, all of which are constituents of various industrial wastes. Analyses on samples taken from the reactor liquors (nearly 1000 samples) include chemical oxygen demand, biological solids concentration, sludge protein content, sludge carbohydrate content, total nitrogen, organic nitrogen, and NH_3-N, individual volatile acids (by gas chromatography), and specific analyses for the various carbon sources employed. In addition, the dissolved oxygen in the reaction liquor was measured (electrometrically) during the period of substrate removal. Also, O_2 uptake was measured for many experiments.

These results will be ready for detailed analysis as soon as spot check runs on some systems have been made. This precautionary procedure prior to detailed analysis and drawing of conclusions has been followed throughout all the research on WP-00325, because we are seeking to delineate basic occurrences common to heterogeneous populations. Therefore, if a phenomenon occurs again and again independently of the random changes in species predominance which occur with heterogeneous populations of sewage origin, we feel that more sound analyses and conclusions can be reached concerning utility of the experiments in the water pollution control field. The results will be analyzed in detail during the coming year. Preliminary analyses indicate that under conditions of nitrogen source abundance in the waste, more metabolic products accumulate in the medium when the COD or carbon source concentration is high in relation to the initial concentration of biological solids, i.e., when substrate removal is due primarily to cell replication. However, these products are metabolized in a later stage of the purification process. Less metabolic products are detected when higher initial biological solids are employed. Under conditions of nitrogen deficiency, a very high amount of metabolic products accumulate when the system is initially at low biological solids concentration, and these products persist in the medium for a very long time; they are only slowly metabolized. At higher sludge concentrations, less products accumulate and they are metabolized more rapidly since there are more "feeders" present.

The types of compounds which were found in the medium included acetic acid (using either glucose, sucrose, sorbitol, or glycerol as the initial exogenous carbon source); propionic acid (using either sorbitol or glycerol as the initial exogenous carbon source); keto-acids (using either glucose, sorbitol, or glycerol as the initial exogenous carbon source, and fructose (especially using sucrose which consists of glucose and fructose; also smaller amounts of fructose were found using glucose and sorbitol as the initial exogenous carbon source).

Some experiments already conducted and others under way at the present time were conducted for exceptionally long aeration periods, much beyond the time required for substrate removal. Some experiments have been carried out for aeration periods as long as 1434 hours. These experiments have allowed assessment of biochemical occurrences under prolonged conditions of endogenous respiration, particularly with regard to products (either lysis products or metabolites elaborated by intact cells) which may appear in the medium during autooxidation of the sludge. This particular aspect has important ramifications to the aerobic digestion of activated sludge. Preliminary analysis of the results indicates that after substrate removal under conditions wherein the nitrogen source for growth was present in non-growth limiting concentrations, the cells rapidly initiate the process of degradation. Intracellular protein is broken down rather rapidly, resulting in release of significant amounts of the ammonia nitrogen which was previously taken up during the period of growth. When the substrate removal was accomplished under conditions of severe nitrogen deficiency (nonproliferating conditions, substrate removed by oxidative assimilation), very little or no ammonia nitrogen was released from the cells during prolonged aeration after the carbon source was assimilated; that is, under this condition, cell protein remained essentially intact. Under conditions of prolonged endogenous respiration following removal of the exogenous carbon source, ribose was found in the medium resulting from the breakdown of some of the cellular nucleic acids. A larger amount of ribose was released by cells which had removed the initial exogenous carbon source under severe nitrogen-deficient conditions. These results require more detailed analysis after spot check runs are made. Also, it should be noted that sufficient samples were taken to establish the kinetic course of appearance of all intermediates and end products. The detailed kinetic analysis of the results will proceed during the coming year.

Studies on the occurrence of metabolic intermediates and/or end products are also under way in a completely mixed continuous flow reactor of the type described in papers 24 and 25 to assess the effect of oxygen tension, or dissolved oxygen concentration, under steady state conditions. These experiments will be completed during the coming year.

G. Studies on the Effect of the Degree of Flocculation on Purification Rate

In the previous report (WP-00325:01-04) the reasons for making investigations into the flocculating ability and effect of polyelectrolytes on the purification capability of activated sludges were given, and studies made up to the time of reporting were summarized. Although no work in this area was proposed in the application for research support on WP-00325 - September 1, 1967, to August 31, 1969, some work was accomplished during the period September 1, 1966, to August 31, 1967, in which polyelectrolyte addition was made to continuous flow reactors. It was found that, for the polyelectrolytes which were used, addition of reasonably economical dosages did not bring about sufficient flocculation of dispersed cells to warrant their use in subsequent continuous flow reactor experiments. Polyelectrolyte additions to systems which were already partially flocculated did seem to enhance greater flocculation. The major purpose of this study and the previous studies on polyelectrolytes (see WP-00325:01-04) was to determine if it would be advantageous to add polyelectrolytes to the reactor to enhance flocculation and settling, thus alleviating some of the operational difficulties encountered in running the many completely mixed reactors employed in the research on the project. Since we did not experience any significant operational advantage and since the polyelectrolytes could conceivably have had some effect (albeit an unknown one at this time) on the kinetics and mechanism of substrate removal and since our previous results were obtained without addition of polyelectrolytes, no further experimentation along these lines was conducted and a decision was made not to add any flocculating agent to the reactor.

It is emphasized that the project work accomplished on polyelectrolytes was entered into in the interests of making a decision pertaining to operational methodology for other experiments conducted on the project. It was not our aim to study polyelectrolyte addition to activated sludge for the purpose of gaining information to be used as a basis for recommending or not recommending their use, modes of addition, selection of polymer or dosage in the field. This aspect is, however, an important and topical subject for research. In the course of performing the experiments accomplished on this project, interesting information concerning polyelectrolyte addition to biological sludges was gained. However, these results have not as yet been subjected to detailed analysis because they were not in the main line of the research effort on WP-00325.

H. Studies on Analytical Technique and Research Methodology

During the course of the research, it has at times been necessary to make rather exhaustive studies pertaining to various chemical analyses and analytical techniques. In cases where significant modification of a biological or chemical technique resulted from these investigations, the information was published or reported at research conferences for scrutiny and possible use by other investigators. In the previous report (WP-00325:01-04), a colorimetric method for determining COD (see papers 1 and 1A) and a statistical study of the spot plate technique for making viable cell counts (see paper 2) were presented in detail. Also, in the detailed summary of work accomplished (see section H of WP-00325:01-04) it was stated that other methods for measuring specific cell components and substrates had already been or were in the process of being investigated. It was found that for some of these analytical techniques there was no need to make significant modifications of the procedures described in the basic chemical or biochemical literature from which the methods were originally taken. For others, some modifications were needed in order to make their use applicable to research in the water pollution control field. In general, those methods have been reported in the materials and methods section of the various papers (1 through 26) which have thus far emanated from the project research. However, we are now in the process of compiling a "methods manual" in order to bring all of the methods together in one laboratory reference manual.

Not all of the concern over methodology involves chemical or biological analytical techniques. For some of the project work it is mandatory that rates of reaction and kinetic constants be calculated. This is exemplified by the calculation of k_1 and L for the exertion of BOD (where such exertion is in accord or can be fitted to first order decreasing rate kinetic equations). One of the interesting undertakings not directly associated with this project has been the compilation of a manual in which sixteen methods for obtaining the BOD constants, k_1 and L, are described, and sample calculations are given. One of these methods was analyzed in considerable detail, since it was desired to use it in analyzing and reporting results for the work presented in paper 20.

The method employed was originally introduced as a procedure for adjusting data which did not show linearity when plotted on semilogarithmic paper. Our investigation of this procedure showed that in addition to its originally intended use, it could be employed to obtain accurate values for first order reaction constants (e.g., k_1 and L for BOD exertion, and k_2 and C_s for reaeration). The method is reported in detail in paper 26.

I. Correlation, Statistical Treatments, Comparison, and Summarization of Project Investigations on WP-00325, WP-00075, and WP-00786

This is a new section which did not appear in the previous progress report (WP-00325:01-04). An important part of the work in the proposal for research support on WP-00325 (September 1, 1967, to August 31, 1969) was the correlative analysis of the results of all studies on this project, and in addition, on WP-00075 and WP-00786. The reasons for the need of such analysis and the benefits derived were delineated in the proposal cited above. The task is, in fact, a tremendous one, since this research proposal and the other two cited above have yielded a great amount of data. The results which have already been published on the work have exerted some significant effects on the mechanistic concepts held in the field, and have spurred the interest of other researchers. The final utility and the magnitude of the contribution to the knowledge of the activated sludge process provided by the completion of these research projects can be greatly enhanced by the correlation and analysis of all of the results. On all of these projects, great care was taken to conduct the experiments under conditions which would allow comparison (e.g., mineral composition of synthetic waste, pH, temperature, air supply, etc.).

Considerable progress has been made in analysis and correlation of data from the three projects during the past year. Values for μ_m and K_s have been cross-checked and can be given with considerable certainty (compare values in papers 16 and 17); also, the μ_m values are verified by the μ_m values given in paper 18. They are, then, reliable values for heterogeneous populations of sewage origin, using a readily available carbohydrate, and are applicable to design of treatment facilities for industrial wastes which require nitrogen supplementation. Since in most studies glucose was used, these values represent, in the main, upper limits for μ_m.

A major effort regarding statistical treatment of all sludge yield results under various conditions and methods of computing sludge yield is nearing completion. The sludge yield is an important parameter which must be estimated as accurately as possible, in order to design sludge handling facilities. etc. Also, in the development and use of completely mixed reactor equations, the sludge yield is an important factor in determining the desired sludge recycle flow and sludge concentration in the recycle flow.

Thus far, over 420 separate determinations of cell yield obtained in studies conducted on the research projects have been gathered together and grouped according to substrate, experimental conditions, etc. To date, substrates for which yield values have been obtained include glucose, sorbitol, galactose, maltose, xylose, mannose, fructose, sorbose, lactose, sucrose, and cellobiose. Yields on other substrates have yet to be analyzed. Yields from batch and continuous flow experiments have been considered separately and jointly. Yield values obtained during shock loading experiments are being analyzed separately. Also the initial biological solids concentration and/or the initial substrate to sludge concentration ratio are taken into consideration in the analysis of the yield data. The means, standard deviations, and coefficients of variance are used to compare the yield values for each group of conditions. Thus far, statistical analysis has been completed for some groups. These include the 200 samples and groups shown in the accompanying table. Detailed discussion and interpretative analysis of the variations in sludge yield and recommendations pertaining to the most reliable values to be used for design will be made after all of the values have been grouped, correlated, and subjected to further statistical analyses.

STATISTICAL ANALYSIS OF SLUDGE YIELD DATA

No.	Experimental Conditions and Substrate	Mean Yield	Standard Deviation	Coefficient of Variance, %	Sample Size
1	Batch, Glucose	0.66	0.11	17	70
2	Batch, Galactose	0.51	0.11	22	9
3	Batch, Maltose	0.51	0.14	28	6
4	Batch, Xylose	0.45	0.10	22	16
5	Batch, Mannose	0.48	0.14	29	4
6	Batch, Fructose	0.54	-	-	2
7	Batch, Sorbose	0.51	0.17	33	13
8	Batch, Lactose	0.50	0.028	6	4
9	Batch, Sucrose	0.64	0.12	19	2
10	Batch, Cellobiose	0.48	0.11	22	4
11	Batch, All Substrates	0.59	0.14	24	133
12	Continuous ($D = 0.25\ hr^{-1}$) Glucose	0.49	0.086	18	31
13	Continuous (All Dilution Rates), Glucose	0.51	0.079	16	69

PROFESSIONAL PERSONNEL

Dr. Anthony F. Gaudy, Jr.

Dr. Anthony F. Gaudy, Jr. is the principal investigator and has devoted from 25 to 40 per cent of his research effort to the accomplishment of this research.

Dr. Elizabeth T. Gaudy

Dr. Elizabeth T. Gaudy is Associate Professor of Microbiology, Microbiology Department, and is not employed on the project, but has devoted some research effort to the conduct of the work (see papers 2, 3, 22, and 23).

Dr. M. Ramanathan

Dr. Ramanathan began his association with the project as a graduate student studying toward the Ph.D. He was associated with the research project from its initiation in September, 1962, through June, 1966, at which time he completed his work. He was formally awarded the Ph.D. degree in August, 1966. The title of his thesis is: "Kinetics of Completely-mixed Activated Sludge." After a visit to his native India, he rejoined the project early in 1967, on an assistant professor-postdoctorate appointment, and has been associated with the project in a responsible capacity since that time.

A number of graduate students have assisted in the conduct of the research; they are listed below:

G. Thabaraj

G. Thabaraj began his doctoral program in January, 1965, and has been associated with the project since that time. His major association with the research project concerns the work on the production, accumulation, and use of metabolic intermediates and/or end products.

K. Peil

K. Peil began his Master's program in February, 1968. His research work is associated with some of the project research; he does not receive any salary from the project.

M. N. Bhatla

M. N. Bhatla began his doctoral program in September, 1961, and was associated with this project from June, 1963, to September, 1964, at which time he completed his work. He was formally awarded the Ph.D. degree in May, 1965. The title of his thesis is: "Studies on the Kinetics and Mechanism of Phasic Oxygen Uptake with Special Regard to the BOD Test."

R. H. Follett

R. H. Follett began his Master's program in June, 1962. He was associated with the work of the project (but received no salary from the project) from September, 1962, to August, 1963, at which time he received his Master's degree. The title of his thesis is: "Studies on the Diphasic Nature of BOD Progression."

F. N. Abu-Niaaj

F. N. Abu-Niaaj began his Master's program in September, 1961. He was associated with research allied to this project and participated in some of the preliminary research which was accomplished prior to initiating the project. He completed his work and received the Master's degree in August, 1962. The title of his Master's report is: "Study of the Velocity of Expression of Biochemical Oxygen Demand."

B. S. Rao

B. S. Rao began his Master's program in January, 1963, and was associated with this project until September, 1964. He received his Master's degree in August, 1964. The title of his thesis is: "Effect of Initial Biological Solids Concentration on Biochemical Response in Discontinuous Activated Sludge Systems."

K. C. Goel

K. C. Goel began his doctoral program in September, 1964, and was associated with the project until the time of completion of his Ph.D. requirements. His Ph.D. degree was granted in May, 1968. The title of his thesis is: "Effects of Ammonia Nitrogen Concentration on Growth of Heterogeneous Populations during Purification of Synthetic Waste Waters."

M. D. Rickard

M. D. Rickard began his doctoral program in June, 1965. His research was associated with the project research; he did not receive any salary from the project. He received the Ph.D. degree in May, 1968. The title of his thesis is: "The Effect of Agitation on the Behavior of Completely Mixed Bacterial Systems."

M. L. Woldman

M. L. Woldman began his Master's program in September, 1965, and was associated with the project research while working toward the Master's degree. He received his Master's degree in May, 1967. The title of his thesis is: "Effects of Selected Organic Polyelectrolytes on Biological Systems."

T. DeGeare

T. DeGeare was employed on this project for six months, during which time he assisted in studies of total cell recycle.

T. D. Yu

T. D. Yu began his Master's program in September, 1964. He accomplished his Master's research in an area related to this project and received his degree in May, 1966. He received no salary from the project. The title of his Master's thesis is: "Effects of Polyelectrolyte Coagulant Aids on Bacterial Cells." After receiving the Master's degree, T. D. Yu began studying toward the doctorate. Portions of his research are related to the project.

WP-00325

LIST OF PUBLICATIONS AND PAPERS
(With Abstracts)

September 1, 1962, to August 31, 1968

Publications and/or papers 1 through 16 cover work on the project from September 1, 1962, to August 31, 1966, and these papers were included in the detailed progress report prepared in August, 1966. In the interest of completeness, they are reported here since some of the publications previously reported as "in press" have since appeared in various journal publications. Copies of the manuscripts for all of these papers were made a part of the progress report cited above, and mailed under separate cover to FWPCA.

September 1, 1966, to August 31, 1968

Publications and/or papers 17 through 26 are included in the present progress report, which covers work completed from September 1, 1966, to August 31, 1968. Copies of these manuscripts are bound in the detailed progress report for this period.

Paper 1

Gaudy, A. F. Jr., and Ramanathan, M., "A Colorimetric Method for Determining Chemical Oxygen Demand," Journal Water Pollution Control Federation, 36, 1479 (1964). Also Proceedings, 3rd Annual Industrial Water and Waste Conference, Rice University, Houston, Texas, June, 1963.

This study, comparing results of COD determinations by the standard titrimetric procedure and by colorimetry, was made using a variety of waste waters. The results indicated that the two procedures yield comparable COD values. The correlation between these methods was very good when the turbidity of the reflux sample was low. Addition of the catalyst at the beginning of the reflux period leads to precipitation of silver chloride in samples containing high chloride concentrations. An alternate procedure wherein the sample was refluxed in the absence of $AgSO_4$ followed by refluxing in its presence was shown to avoid the formation of a precipitate.

A copy of this paper is not included, since Paper 1A contains the same information plus additional data.

Paper 1A

Gaudy, A. F. Jr., and Ramanathan, M., "A Colorimetric Method for Determining Chemical Oxygen Demand," Proceedings, 19th Industrial Waste Conference, Purdue University, 1964, p. 915.

This paper was presented under the same title as Paper 1, but included additional results in which the work was extended to cover a wider variety of waste waters. The paper was presented at the Purdue Industrial Waste Conference at the specific request of Professor Bloodgood.

Paper 2

Gaudy, A. F. Jr., Abu-Niaaj, F., and Gaudy, E. T., "Statistical Study of the Spot-plate Technique for Viable Cell Counts," Appl. Microbiol., 11, 305 (1963).

A statistical study of the spot-plate technique was made for the purpose of establishing an acceptable counting range. The organism used in these studies was Micrococcus lysodeikticus. Standard deviations and coefficients of variation were computed for counts ranging from 20 to 440. The lower and upper limits for acceptable counts, based on coefficients of variation of 10 and 5.8, respectively, were chosen as 100 and 300.

Paper 3

Gaudy, A. F. Jr., Bhatla, M. N., and Gaudy, E. T., "The Use of Chemical Oxygen Demand Values of Bacterial Cells in Waste Water Purification," Appl. Microbiol., 12, 254 (1964).

Four methods for determining substrate recoveries in studies concerned with the partition of substrate between sludge synthesis and respiration were investigated. An energy balance, comparing chemical oxygen demand (COD) removed with the summation of oxygen uptake and the COD of the cells produced, yielded average recoveries closer to 100 per cent than any of the other three methods tested. The standard COD test was shown to yield highly reproducible values when used to determine the COD of activated sludge. Although the protein and carbohydrate content of the cells varied with cell age, a concomitant variation in cell COD was not noted.

Paper 4

Gaudy, A. F. Jr., Bhatla, M. N., and Abu-Niaaj, F., "Studies on the Occurrence of the Plateau in BOD Exertion. I. Heterogeneous and Pure Culture Seeds with Various Concentrations of Glucose as Carbon Source." (Portions of this paper were presented at the 18th Industrial Waste Conference, Purdue University, 1963.)

There had been an increasing number of conflicting reports concerning the occurrence of a plateau in oxygen uptake during the exertion of biochemical oxygen demand. This mechanistic aspect of substrate removal is of great significance in determining the kinetics of waste water purification by activated sludge, and is also important from the standpoint of analyzing the results of the BOD test. It was essential to determine the conditions under which the plateau could be manifested, and hopefully to gain some insight into the causative mechanisms for its occurrence. The studies herein reported were undertaken primarily to determine the generality or constancy of the occurrence of the plateau with various seeding conditions and substrate concentrations in the BOD bottle. In these studies the carbon source consisted of glucose. The results showed with reasonable certainty that the plateau in oxygen uptake during exertion of carbonaceous BOD occurs using various seeding materials and glucose as the carbon source. Analysis of the oxygen uptake data and bacterial count data taken at each sampling point for DO provided a clear indication that the rapid rise in oxygen uptake (first stage) corresponded to rapid bacterial multiplication. The plateau corresponded to the peak range of population density (the stationary phase); the second stage of O_2 uptake corresponded to a decreasing portion of the bacterial count curve.

Paper 5

Gaudy, A. F. Jr., and Bhatla, M. N., "Studies on the Occurrence of the Plateau in BOD Exertion. II. Experiments using Different Carbohydrates and Multicomponent Systems." (Portions of this paper were presented at the 18th Industrial Waste Conference, Purdue University, May, 1963.)

The results presented herein represent an extension of the work reported in Paper 4. In these studies the work was extended to include a variety of compounds using young heterogeneous populations acclimated to the particular compound under study, and to multicomponent substrate systems using both young heterogeneous populations and a pure culture seed. The substrates employed included glycerol, glycogen, lactose, ribose, glutamate, sorbitol, melibiose, galactose, and various combinations of these compounds with glucose. The work indicated that the plateau could occur with a variety of carbon sources, and that the duration or severity of the plateau corresponded approximately to the length of the stationary phase and the region of maximum population. Based upon these studies, four related theories which could possibly explain the occurrence of the plateau were formulated.

Paper 6

Gaudy, A. F. Jr., Bhatla, M. N., and Abu-Niaaj, F., "Studies on the Occurrence of the Plateau in BOD Exertion," Proc. Industrial Waste Conference, Purdue University, XLVIII, 183, May, 1963.

Portions of the results presented in Papers 4 and 5 are combined in this paper.

Paper 7

Follett, R. H., and Gaudy, A. F. Jr., "Studies on the Occurrence of the Plateau in BOD Exertion. III. Simple and Complex Wastes with Various Seed and Substrate Concentrations," Proceedings, 14th Oklahoma Industrial Wastes Conference, Oklahoma Water and Pollution Control Association, 1963, p. 346.

This paper represents an extension of the work reported in Papers 4 and 5, and includes investigations of the effects of substrate concentration and the size of the initial seed inoculum on the occurrence of the plateau in BOD exertion. Also included were studies on the occurrence of the plateau using complex media and whole wastes. Phasic oxygen uptake in the BOD bottle occurred in all systems tested; however, the plateau between phases was not evidenced for nutrient broth.

Paper 8

Gaudy, A. F. Jr., Bhatla, M. N., Follett, R. H., and Abu-Niaaj, F., "Factors Affecting the Existence of the Plateau during the Exertion of BOD," J. Water Pollution Control Fed., 37, 444 (1965).

In this paper the results reported in Papers 4, 5, and 7 were summarized for journal publication. In summary, at this point of the investigation it was concluded that the occurrence of the plateau in BOD exertion appeared to be independent of the type of seed employed. The plateau was exhibited over a wide range of substrates. It occurred in both single carbon source systems and in systems containing a complex mixture of organic compounds. Within the usual limits of seed concentration employed in the BOD test, the occurrence of the plateau was not dependent upon seed concentration. The relation between oxygen uptake and viable bacterial count remained consistent throughout the investigation, and four theories of causation for the occurrence of the plateau were derived, based upon evidence thus far obtained.

Paper 9

Bhatla, M. N., and Gaudy, A. F. Jr., "Studies on the Causation of Phasic Oxygen Uptake in High Energy Systems," Proceedings, 19th Industrial Waste Conference, Purdue University, 1964, p. 871 (J. Water Pollution Control Fed., in press, 1966).

Studies were undertaken using heterogeneous populations developed on a glucose-salts medium from an initial sewage seed for the purpose of examining possible causative mechanisms for the occurrence of the plateau in BOD exertion. In these studies substrate and cell concentrations much greater than those employed in the BOD bottle were used. The studies included investigations on the effect of substrate concentration and initial solids concentration on the occurrence of the plateau using both sewage seed and acclimated seed. In addition, long term aeration studies were made to determine possible relationships between phases of oxygen uptake and substrate removal, intracellular protein content, and solids production. Efforts were also made to determine whether carbon sources other than the original carbon source were excreted into the medium during the plateau or the second stage of oxygen uptake. It was found for substrate concentrations up to 1000 mg/l that there was no distinct or critical substrate concentration at which the plateau could not be observed. The results did indicate, however, that the phases gradually merge, and that the plateau tends to become indistinct and may finally disappear as the total energy of the system increases. From these studies it appeared that the release of cellular components due to lysis of a portion of the bacterial population after reaching the maximum attainable numbers and the need for an acclimation period before metabolism of these released components could not be cited as a general causative mechanism for a plateau in BOD exertion by heterogeneous populations. Also, in these particular studies it was found that the release of intermediates or end products during the metabolism of the original exogenous substrate could not be cited as a causative mechanism contributing to the existence of a plateau for heterogeneous populations.

Paper 10

Bhatla, M. N., and Gaudy, A. F. Jr., "Studies on the Plateau in Oxygen Uptake during Exertion of Biochemical Oxygen Demand by Pure Cultures of Bacteria," Biotech. and Bioeng., VII, 387 (1965).

Evidence for generation of the plateau in oxygen uptake during exertion of carbonaceous BOD by pure cultures was sought by making long-term Warburg studies using glucose and phthalic acid as substrates. One organism, tentatively identified as Escherichia intermedia, of the fifty-seven tested, exhibited a plateau. Detailed studies on this organism indicated that generation of the plateau depended upon the type of substrate and, to some extent, upon the initial cell concentration employed, but not upon substrate concentration. Oxygen utilization during the second stage was accompanied by metabolism of secondary extracellular carbon source(s) produced by these cells during metabolism of the original extracellular carbon source (glucose) in the first phase of O_2 uptake. The secondary substrate was registered as volatile acids. Selective inhibition of protein synthesis at various times during oxygen uptake led to the conclusion that the plateau occurred because of the need for an induction period before the secondary substrate could be metabolized. Using this organism, it was also found that a plateau could be generated during endogenous respiration. This secondary oxygen utilization was accompanied by uptake of organic materials excreted into the medium by the cells during metabolism of endogenous carbon sources.

Paper 11

Bhatla, M. N., and A. F. Gaudy, Jr., "Sequential Substrate Removal in a Dilute System using Heterogeneous Microbial Populations," Appl. Microbiol., 13, 345 (1965).

The sequential metabolism of substrates by heterogeneous bacterial populations has been previously reported from this laboratory in studies using high substrate concentrations. This phenomenon has now been shown to occur at very dilute substrate concentrations, i.e., 5 mg/l glucose + 5 mg/l sorbitol, in studies conducted under the conditions of the standard biochemical oxygen demand (BOD) test. Sequential metabolism of these substrates resulted in a diphasic curve of accumulated oxygen uptake, wherein the two phases were separated by a discernible plateau. These findings illustrate one possible explanation for the generation of discontinuity in the kinetic course of carbonaceous BOD exertion.

Paper 12

Bhatla, M. N., and Gaudy, A. F. Jr., "The Role of Protozoa in the Diphasic Exertion of BOD," Journal Sanitary Engineering Division, Proceedings ASCE, 91, SA3 (4371), 63 (1965).

Causation for the occurrence of a plateau in diphasic BOD curves was investigated for heterogeneous seeds of sewage origin. Oxygen uptake studies were conducted in discontinuous systems using glucose synthetic waste, employing manometric techniques as well as standard BOD dilution procedure. Bacterial and protozoan population growth patterns were followed during the course of oxygen utilization. The effect of the protozoa-bacteria relationship on the kinetics of oxygen uptake and the occurrence of the plateau was examined. In addition, the effect of inoculations of protozoa to bacterial systems was studied with respect to oxygen demand and other biochemical parameters. Pasteurization and microfiltration of the seed material were employed to exclude protozoa from the bacterial population in some studies. Chloramphenicol was used to delineate the role of bacterial enzyme synthesis in generating the plateau and the second stage of oxygen uptake.

It was found that for heterogeneous populations the second stage of oxygen uptake was due almost entirely to protozoan metabolism, and that the length of the plateau was related to the relative numbers of bacteria and protozoa in the seed material. When the plateau occurred, it corresponded to the range of maximum viable bacterial count, and the secondary rise in oxygen uptake was marked by rapid multiplication of the protozoa; approximately fifty per cent of the second stage oxygen uptake had been exerted when the protozoan population reached maximum density. It was found that for heterogeneous populations a two-phase oxygen uptake curve during exertion of carbonaceous BOD is caused by the sequential growth of two segments of the population, i.e., the growth of bacteria, removing the exogenous carbon source, and the growth of the protozoa, using the bacteria produced in the first stage as food. .In contrast to prevalent concepts in the field, the protozoa were found to play a direct and autonomous role in the exertion of carbonaceous biochemical oxygen demand.

Paper 13

Rao, B. S., and Gaudy, A. F. Jr., "Effect of Sludge Concentration on Various Aspects of Biological Activity in Activated Sludge," J. Water Pollution Control Fed., 38, 794 (1966); also Proceedings, 5th Industrial Waste Conference, Texas Water Pollution Control Association, 1965, p. 216.

There have been various and somewhat conflicting reports in the literature concerning the relationship between sludge concentration and substrate removal rate for activated sludge systems. It has been reported that a simple linear relationship between the rate of substrate removal and initial sludge concentration exists, and that the relation is dependent upon the type of carbon source or type of waste being metabolized. The implication was that the slope of the linear relationship was specific for a particular type of waste. Also, there have been many conflicting reports pertaining to the sludge yield. Here again the implication is that the sludge yield is determined by the type of substrate or waste or its free energy content. Although it would be ideal from an engineering standpoint if such relationships were entirely substrate-dependent, there were no published data to show that the relationship remains constant over a prolonged period of operation. It was felt that a considerable insight into the constancy of the cell yield and the relation between substrate removal rate and initial solids concentration could be obtained by conducting an integrated long-term study under highly controlled operational conditions using only one carbon source. Both aspects have an important bearing on the kinetics and mechanism of waste water purification by the activated sludge process. The long-term studies indicated that there is a straight line relationship between initial solids concentration and substrate removal rate for a specific sludge. However, for sludge samples taken at different times from a unit operated over a long period, the following observations were made:

1. The relationship between initial solids concentration and rate of COD removal varies for a single substrate.

2. The cell yield varies for a single substrate.

3. The percentage of the theoretical oxygen demand exerted at the point of both glucose and COD removal decreases as initial solids concentration increases.

The variations in the relationship between initial biological solids concentration and purification rate and in cell yield appear to be due to changes in predominance in the population which occur in spite of controlled, constant operational conditions. The decrease in percentage theoretical oxygen demand exerted at substrate removal when initial solids are increased can be explained on the basis of differences in the amount of the total substrate utilized in early synthesis of carbohydrates. This synthesis, which occurs when starved cells are placed in fresh medium, requires less energy and therefore consumes less oxygen than do synthetic processes which take place after the cells have become adjusted to the new environment.

Paper 14

Komolrit, K., Goel, K. C., and Gaudy, A. F. Jr., "Regulation of Exogenous Nitrogen Supply and Its Possible Applications to the Activated Sludge Process," Journal Water Pollution Control Federation, 39, 251, 1967.

This paper was presented at the 38th Annual Conference of the Water Pollution Control Federation, Atlantic City, New Jersey, October, 1965.

Work was undertaken to determine the effect of controlled periodic addition of nitrogen on the treatment of nitrogen-deficient synthetic waste by activated sludge. Batch activated sludges were developed (24-hour feeding cycle; 1/3 sludge wasting), using glucose as carbon source and ammonium sulfate as the source of nitrogen. After a period of balanced operation, the organic loading was continued but nitrogen was withheld. After several such feeding periods during which no sludge was wasted, the sludge was parted and its metabolic characteristics were studied under endogenous conditions, with addition and without addition of nitrogen source. These sludges were then washed and subjected to substrate loadings in the presence and absence of a nitrogen source. During all phases of study the partition between respiration and synthesis was compared in the presence and the absence of nitrogen. The parameters investigated included COD removal, glucose removal, oxygen uptake, and biological solids production. Sludge composition (carbohydrate and protein) was also determined in all experiments to gain information on the biochemical nature of the sludge and changes occurring under the various operational conditions studied.

It was found that the sludge retained an ability to remove the carbon source for some time in the absence of an exogenous nitrogen source when the loading was applied at 24-hour intervals. However, the activity of the sludge, as measured by the COD removal rate and respiration rate, gradually decreased as the cell age increased. In the absence of an exogenous nitrogen source, the substrate was channelled mainly into carbohydrate synthesis. The decrease in sludge activity was related to an accumulation of synthesized carbohydrate and a decrease in protein content as the sludge age increased. However, the activity of this high carbohydrate-containing sludge could be considerably restored upon addition of nitrogen under endogenous conditions. The addition of the nitrogen source also resulted in a significant increase in the endogenous respiration rate of the sludge, and in the internal conversion of the stored carbohydrate to protein. The increased activity accompanying this change in chemical composition of the sludge was manifested by a significant recovery in substrate removal rate and respiration rate upon re-feeding with organic waste in the absence of an exogenous source of nitrogen.

The results of the study suggest that the activity of activated sludge is governed largely by its protein content, and that the nitrogen requirement of activated sludge may be supplied intermittently or only in the endogenous stage rather than simultaneously with the organic loading. A possible flow sheet incorporating aspects of biosorption and extended aeration was presented. Such operation may result in considerable savings in nitrogen and decreased opportunity for mineral nutrition problems in the receiving stream.

Paper 15

Krishnan, P., and Gaudy, A. F. Jr., "Mechanism and Kinetics of Substrate Removal at High Biological Solids Concentrations," Proceedings, 21st Purdue Industrial Waste Conference, Purdue University, May, 1966; also, Journal Water Pollution Control Federation, 40, R50, 1968.

In a previous paper, Rao and Gaudy (see paper 13) presented a theory based upon experimental findings which explained why the percentage of the theoretical oxygen demand exerted at the time of substrate removal should decrease as initial biological solids concentration was increased. However, the highest biological solids concentration employed in the previous work was approximately 2000 mg/l, and it was felt that further studies at higher solids concentrations were warranted. In addition, work at higher solids concentrations permitted investigations into the amount of substrate which could be immediately taken up by the sludge. Since very rapid purification is accomplished only at high solids concentrations, the present study offered an opportunity to gain further insight into the possible roles of adsorption and biological assimilation during rapid substrate removal. The work is particularly pertinent to the biosorption process, and to the applicability of a process modification recently reported by Komolrit, Goel, and Gaudy (see paper 14). It was found that the sludge yield for initial solids concentrations up to 5000 mg/l was in the general range of those previously found at 2000 mg/l and below. Concerning the biochemical mechanism of substrate removal, the work which formed the basis of the theory presented by Rao and Gaudy at lower solids concentrations was substantiated at the high solids concentrations used in the present study. When the initial solids level was high, almost all of the substrate could be removed before an appreciable amount of protein was synthesized. It is to be expected that even when starting with low initial biological solids concentrations, carbohydrate synthesis precedes protein synthesis; however, there are so few cells in the system that the early synthesis of carbohydrate does not result in appreciable substrate removal. In this case, substrate removal actually does depend on balanced growth, i.e., replication of the cells; whereas in high solids systems it does not. Therefore, the solids level affects the mechanism by which the substrate is removed. Under conditions of high solids, where little or no protein is made during the period of substrate removal, it would be expected that purification would not be dependent upon a source of nitrogen. The ability of high solids systems to remove substrate in the complete absence of protein synthesis was rigorously demonstrated by inhibiting protein synthesis with chloramphenicol. The experimental data demonstrating the initial conversion of substrate to carbohydrate also made it possible to offer a sound biochemical explanation for the approximately zero order kinetics of substrate and COD removal, oxygen uptake, and solids production often observed with high solids systems. Since these systems function initially as nonproliferating systems, they would be expected to exhibit kinetics typical of such systems, i.e., linear or zero-order kinetics. The results of experiments using sorbitol as substrate throw particular light on the question of adsorption as a primary mechanism of substrate removal. Sorbitol does not react to the anthrone test, and therefore it does not register as carbohydrate. However, during substrate removal, the carbohydrate content of the sludge increased rather rapidly. This finding indicates that the removal of sorbitol was the result of its biochemical conversion into carbohydrate, thus providing evidence against the adsorption mechanism.

Paper 16

Gaudy, A. F. Jr., Ramanathan, M., and Rao, B. S., "Kinetic Behavior of Heterogeneous Populations in Completely Mixed Reactors." This paper was presented at the 152nd National Meeting, American Chemical Society, New York, September, 1966. Biotechnology and Bioengineering, IX, 387, 1967.

Long term investigations of the kinetic behavior of heterogeneous microbial populations were undertaken in continuous flow completely mixed reactors operated at various dilution rates. Glucose was used as the growth-limiting nutrient. The physiological growth parameters for cells harvested from continuous flow reactors were determined using batch experiments. It was found that the growth parameters, maximum growth rate (μ_m), saturation constant (k_s), and cell yield (Y), varied for each dilution rate, and cannot be considered as precise constants in depicting the kinetic behavior of heterogeneous populations. In addition, it was found that the yield coefficients obtained from batch experiments were always lower than those obtained from continuous flow experiments. Levels of substrate and biological solids calculated for different dilution rates using growth constants from batch experiments did not agree with the experimental values observed in steady state experiments. However, when the yield values from the continuous flow experiments were used in conjunction with batch values for μ_m and k_s, the theoretical and experimental dilute-out curves agreed fairly closely (within the range needed for engineering prediction) until the culture began to wash out of the unit. In general, the data substantiated the use of the single phase relationship between growth rate and substrate concentration described by the Monod equation,

$$\mu = \frac{\mu_m S}{k_s + S}$$

Paper 17

Ramanathan, M., and Gaudy, A. F. Jr., "Effect of High Substrate Concentration and Cell Feedback on Kinetic Behavior of Heterogeneous Populations in Completely Mixed Systems." (Submitted for publication in Biotechnology and Bioengineering, 1968.)

Growth kinetics of heterogeneous populations of sewage origin were studied in completely mixed reactors of the once-through type at a high concentration of incoming substrate, 3000 mg/l glucose, and in systems employing cell feedback or sludge recycle at an incoming substrate concentration of 1000 mg/l glucose. The recycle flow rate employed was 25 per cent of the incoming feed flow, and the concentration of cells in the recycle was maintained as closely as possible at 150 per cent of the cell concentration in the reactor. Studies were made at various dilution rates. Throughout these studies, batch experiments using cells grown at the various dilution rates were made to determine k_s and μ_m values. As in previous studies using heterogeneous populations, the relationship between specific growth rate, μ, and substrate concentration, S, was represented better by the Monod equation than by any other which was tested. The growth "constants" μ_m, k_s, and Y were found to fall in the same general range as those determined in previous studies in once-through systems operated at 1000 mg/l glucose. It was observed that cell recycle, even at the relatively low concentration factor employed in these studies, greatly enhanced the flocculating and settling characteristics of the cells.

__Paper 18__

Goel, K. C., and Gaudy, A. F. Jr., "Regulation of Nitrogen Levels for Heterogeneous Populations of Sewage Origin Grown in Completely Mixed Reactors." (Submitted for publication in Bioengineering and Biotechnology, 1968.)

Heterogeneous populations of sewage origin were grown continuously at dilution rates from 1/12 hr^{-1} to dilute-out (1/1 hr^{-1}) using glucose (1000 mg/l) as carbon source, and three concentrations of ammonia nitrogen as the nitrogen source (COD/N = 70/1, 40/1, and 25/1). The effects of nitrogen level and growth rate (dilution rate) on substrate removal, biological solids production, cellular carbohydrate and protein, and ammonia nitrogen in the effluent were examined. It was found that the optimum level of nitrogen supplementation for the "synthetic nitrogen-deficient waste" employed should not be based solely on the desired effluent quality with respect to COD removal, but should include due consideration of reactor detention time (or dilution rate), and the allowable (or desirable) level of nitrogen leakage in the effluent.

Paper 19

Goel, K. C., and Gaudy, A. F. Jr., "Studies on the Relationship between Specific Growth Rate and Concentration of Nitrogen Source for Heterogeneous Microbial Populations of Sewage Origin." (Submitted for publication in Biotechnology and Bioengineering, 1968.)

Batch experiments were run using heterogeneous populations to determine whether a hyperbolic equation of the type suggested by Monod could be used to predict the relation between specific growth rate, μ, and ammonia nitrogen concentration when ammonia nitrogen was the growth-limiting nutrient. The heterogeneous populations employed were developed from sewage seed and grown on glucose at various levels of nitrogen and various dilution rates in completely mixed continuous flow reactors. It was found that the hyperbolic function could be used. Values of μ_m in the range of 0.4-0.7 hr^{-1} were observed and values of K_s, in general, ranged from 1.5 to 4.0 mg/l. Variation in the values of these growth "constants" did not follow any discernible pattern related to past growth history (i.e., COD/N ratio or dilution rate at which the cells were previously grown).

Paper 20

Isaacs, W. P., and Gaudy, A. F. Jr., "Comparison of BOD Exertion in a Simulated Stream and in Standard BOD Bottles." Proceedings, 22nd Purdue Industrial Waste Conference, Purdue University, May, 1967.

The exertion of biochemical oxygen demand was studied in a simulated receiving stream apparatus and compared to BOD exertion using the standard BOD bottle technique. It was shown that phasic exertion of biochemical oxygen demand occurs in turbulent systems under physical conditions similar to those which exist in receiving streams. The dissolved oxygen concentration curve in the simulated receiving stream (sag curve) could be reproduced using three distinct kinetic phases and rate constants derived from oxygen uptake data obtained in the simulated stream. It was also shown that data obtained in BOD bottles could be used to make a reasonable prediction of the observed minimum or critical DO, provided that the seed concentration in the BOD bottle was identical, or nearly so, to the biological solids concentration in the receiving stream at the point of introduction of the synthetic waste, and provided that a phasic, rather than one continuous first order, expression was employed to describe BOD exertion. It is significant that no plateau was actually observed in the BOD bottle data which were used in making the prediction of the sag curve in the simulated river system, but a fairly accurate prediction could be made by estimating the extent of the first stage exertion from a knowledge of its extent in systems which did exhibit a plateau marking the end of the first stage O_2 uptake. The results of this experimentation indicate that although much more work is needed to delineate factors affecting the various rate constants and to define more closely the mechanisms which govern the kinetic expression of oxygen uptake, the approach taken in this work offers some promise of providing adequate prediction of the course of oxygen depletion (due to exertion of BOD) and recovery in receiving streams.

Paper 21

Goel, K. C., and Gaudy, A. F. Jr., "Regeneration of Oxidative Assimilation Capacity by Intracellular Conversion of Storage Products to Protein." (Submitted for publication in Applied Microbiology, 1968.)

It was known from previous studies that the capacity of a heterogeneous biological population for oxidative assimilation of glucose could be renewed by periodically subjecting the sludge (or a portion thereof) to endogenous respiration in the presence of an exogenous source of nitrogen. Further study of this process led to a suggested modification of the activated sludge process for nitrogen-deficient waste. However, it was not known whether renewal of oxidative assimilation capacity was possible on substrates which require the presence of inducible enzyme(s), or on substrates which were not carbohydrate. Therefore, studies were designed using lactose and acetate as carbon sources. Both carbon sources were removed under conditions of oxidative assimilation and the storage products (or a portion thereof) were converted into protein when the sludge was subjected to a period of endogenous respiration (with respect to carbon source) in the presence of an exogenous supply of ammonium sulfate. The "regenerated" sludge exhibited a renewed capacity for oxidative assimilation, thereby indicating that requisite inducible enzyme(s) (e.g., β-galactosidase in the case of lactose, isocitritase and malate synthetase in the case of acetate) were not diluted out in the endogenous phase to a degree sufficient to hamper renewed oxidative assimilation capacity. The results also indicated that a non-carbohydrate carbon source could be successfully removed from the medium using this process. However, in the case of acetate, the oxidative assimilation capacity after "regeneration" was not quite as high as the initial capacity.

Paper 22

Gaudy, A. F. Jr., Goel, K. C., and Gaudy, Elizabeth T., "Studies on Continuous Oxidative Assimilation of Acetic Acid and Endogenous Protein Synthesis Applicable to Treatment of Nitrogen-deficient Wastewaters." (Submitted for publication in Applied Microbiology 1968.)

Previous study indicated that the oxidative assimilation capacity of a heterogeneous microbial population for acetic acid did not return to the initial value after a period of endogenous respiration in the presence of ammonium sulfate. This finding provided cause for restraint in recommending the possible use of a continuous oxidative assimilation process for the treatment of nitrogen-deficient wastewaters of noncarbohydrate nature. In order to put the process to a severe test, a laboratory scale pilot plant study was made. Acetate (1000 mg/l) was fed continuously to a completely mixed aerated reactor vessel from which the mixed liquor was channelled to a settling basin. The settling basin supernatant was continuously discharged, and a portion of the settled biological sludge was subjected to endogenous respiration in the presence of ammonium sulfate and recycled to the aeration vessel. Experiments were conducted at three levels of ammonia nitrogen in the endogenous phase. It was found that approximately 90% removal of the carbon source (expressed as chemical oxygen demand, COD) was attained using a low level of supplemental nitrogen (COD/N = 70/1) and a fairly low reactor detention time (4 hours). Based upon these and previous results, it has been concluded that the process shows promise for application to the treatment of a wide variety of nitrogen-deficient industrial wastes.

Paper 23

Gaudy, A. F. Jr., Goel, K. C., and Gaudy, Elizabeth T., "Application of Continuous Oxidative Assimilation and Endogenous Protein Synthesis to the Treatment of Carbohydrate Wastes Deficient in Nitrogen." (Submitted for publication in Biotechnology and Bioengineering, 1968.)

A synthetic waste (with glucose as carbon source) devoid of a source of nitrogen was purified in a laboratory scale pilot plant by a new modification of the activated sludge process. The process makes use of a separate carbon assimilation (oxidative assimilation) phase and an endogenous phase in which ammonia is added to a portion of the settled sludge and non-nitrogenous products stored in the cells in the assimilation phase are converted to protein. It was found that sludge so treated could, when recycled to the assimilation tank, carry out continuous oxidative assimilation of the waste. Various COD/N ratios were studied. At the highest, 70/1, 90% purification efficiency was achieved.

Paper 24

Rickard, M. D., and Gaudy, A. F. Jr., "Effect of Mixing Energy on Sludge Yield and Cell Composition for Heterogeneous Populations in Completely Mixed Systems."

This paper was presented at the 39th Annual Conference of the Water Pollution Control Federation, New York City, New York, October, 1967. Journal Water Pollution Control Federation (in press) 1968.

Experiments were run in a completely mixed continuous flow reactor in which the degree of agitation of the mixed liquor could be varied in a controlled manner. The parameter used to assess the degree of agitation of the mixed liquor was the velocity gradient, G. The primary purpose of conducting this experimentation was to determine whether the degree of agitation in the system had an effect upon the biological solids yield, the substrate removal efficiency, and the oxygen utilization of the cells. Oxygen uptake was measured continuously using a Beckman gas analyzer. It was found that both the biological solids concentration (or biological solids yield) and oxygen uptake varied with the amount of agitation provided. There was a decrease in cell yield and an increase in O_2 uptake with increasing turbulence or agitation of the system; the decrease in cell yield was observed to occur primarily at the expense of the carbohydrate content of the cells. This resulted in the production of a sludge having a higher protein content at the higher agitation rates. Of the five possible mechanisms which could be employed to explain the results, it appeared that the increased oxygen uptake with increased turbulence most likely resulted from reductions in the cell-liquid interfacial resistance to oxygen transfer as the amount of mixing energy was increased.

Paper 25

Rickard, M. D., and Gaudy, A. F. Jr., "Effect of Oxygen Tension on O_2 Uptake and Sludge Yield in Completely Mixed Heterogeneous Populations." Presented at the 23rd Industrial Wastes Conference, Purdue University, May, 1968.

Experiments were run in a completely mixed continuous flow reactor operated with a constant degree of agitation (i.e., velocity gradient, G), and the percentage of oxygen in the aerating gas was varied. The purpose of these experiments was to determine whether increasing or decreasing the dissolved oxygen concentration of the mixed liquor at a constant degree of agitation in the system would affect the biological solids yield and oxygen uptake. The system was run under steady state conditions at various levels of dissolved oxygen concentration between 1.4 and 7.1 mg/l. It was found that the dissolved oxygen concentration had no effect on oxygen uptake or on biological solids concentration. In addition, there was no effect on the amount of RNA or protein contained in the cells. As oxygen concentration was increased, there was a slight increase in DNA content of the cells and a small improvement in both COD and glucose removal efficiency. There was a significant decrease in carbohydrate content of the cells. Concerning the oxygen uptake, it was concluded, based upon the present results and work previously accomplished at varying agitation rates in the system, that agitation exerts an effect independent of oxygen tension at dissolved oxygen concentrations above accepted values for critical DO concentration.

Paper 26

Isaacs, W. P., and Gaudy, A. F. Jr., "A Method for Determining Constants of First Order Reactions from Experimental Data." Biotechnology and Bioengineering, X, 69-82, 1968.

The authors have utilized a previously proposed mathematical equation (introduced originally for development of empirical equations) as a useful tool for evaluation of first-order reaction rate constants. By assigning physical significance to the parameter α , the equation can be utilized in obtaining excellent estimates for limiting boundary values and velocity constants.

MANUSCRIPTS OF PAPERS AND PUBLICATIONS
ON RESEARCH SUPPORTED BY
GRANT WP-00325

(Sept. 1, 1966, to Aug. 31, 1968)

For papers 1 through 16 see Progress Report on WP-00325 for September 1, 1962, to August 31, 1966.

PAPER 17

Ramanathan, M., and Gaudy, A. F. Jr., "Effect of High Substrate Concentration and Cell Feedback on Kinetic Behavior of Heterogeneous Populations in Completely Mixed Systems." (Submitted for publication in Biotechnology and Bioengineering, 1968.)

EFFECT OF HIGH SUBSTRATE CONCENTRATION AND CELL FEEDBACK ON KINETIC BEHAVIOR OF HETEROGENEOUS POPULATIONS IN COMPLETELY MIXED SYSTEMS

By

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SUMMARY

Growth kinetics of heterogeneous populations of sewage origin were studied in completely mixed reactors of the once-through type at a high concentration of incoming substrate, 3000 mg/l glucose, and in systems employing cell feedback or sludge recycle at an incoming substrate concentration of 1000 mg/l glucose. The recycle flow rate employed was 25 per cent of the incoming feed flow, and the concentration of cells in the recycle was maintained as closely as possible at 150 per cent of the cell concentration in the reactor. Studies were made at various dilution rates. Throughout these studies, batch experiments using cells grown at the various dilution rates were made to determine k_s and μ_m values. As in previous studies using heterogeneous populations, the relationship between specific growth rate, μ, and substrate concentration, S, was represented better by the Monod equation than by any other which was tested. The growth "constants" μ_m, k_s and Y were found to fall in the same general range as those determined in previous studies in once-through systems operated at 1000 mg/l glucose. It was observed that cell recycle, even at the relatively low concentration factor employed in these studies, greatly enhanced the flocculating and settling characteristics of the cells.

INTRODUCTION

In a previous report, the results of studies on growth characteristics of heterogeneous microbial populations cultivated continuously in completely mixed reactors of the once-through type (no cell feedback) were presented (1). In those systems the carbon source (1000 mg/l glucose) was the growth-limiting nutrient.

The work was accomplished in order to determine whether kinetic theory which has proven useful in the study of pure culture systems (2) could be usefully applied for natural microbial populations, and to determine possible interpretative limitations which should be placed upon the kinetic theory if it applied to such populations. From these studies it was possible to conclude that the single phase relationship between growth rate and substrate concentration embodied in the "Monod" equation is applicable to mixed populations, although the kinetic constants are subject to variation partially because of the selective pressure exerted by the dilution rate. Also, these systems were found to be subject to changes in predominant species at a constant dilution rate; consequently, a "steady state" with respect to the biomass could be only approached, not attained. Some suggestions or guidelines pertaining to the use of such systems in obtaining design and operational criteria were also presented. Because of the potential importance of the kinetic approaches which were examined for useful application in pollution control work and because it was desirable to gain further insight into the kinetic behavior of natural populations, additional study seemed warranted. The present report represents an extension of the previous work to include studies at higher substrate concentrations and experimentation in which cell feedback (sludge recycle) was practiced.

THEORETICAL CONSIDERATIONS

Various kinetic models relating μ and S, which are pertinent to the present experimentation, have been presented in the literature. These were reviewed and evaluated experimentally in the previous report (1). Also development of the steady state equations for reactors of the once-through type were presented using the notation of Herbert (3). The development of kinetic formulation for systems employing cell recycle is somewhat more entailed, but may be approached in the same manner as for once-through systems. Since development of equations for cell recycle systems has been presented by Herbert (3), they need be reviewed only briefly here. In the following development, the notation of Herbert is used with minor modifications in terms where clarification seemed warranted.

A typical flow diagram for the completely mixed activated sludge process employing "controlled" cell feedback is shown in Figure 1. The notation on the flowsheet is defined in the development of the following operational equations.

The steady state values of biological solids and substrate concentrations in the reactor (and effluent) can be determined by writing a materials balance around the reactor. The rate of change in the amount of cells present in the system is equal to the sum of the rates of increase in cells due to inflow, recycle, and growth minus the rate of removal of cells from the reactor:

Increase = inflow + recycle + growth - outflow

$$V \frac{dx}{dt} = F x_i + \alpha F c x + \mu x V - (1 + \alpha) F x \qquad (1)$$

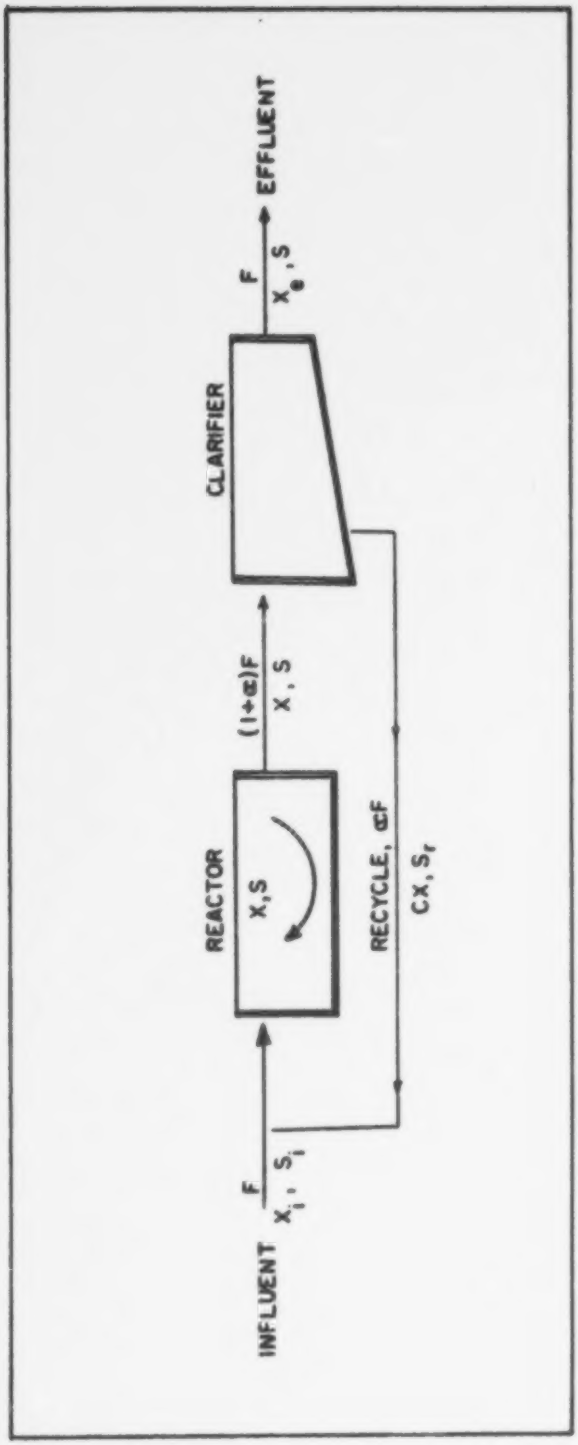
where

V = volume of reactor

$\frac{dx}{dt}$ = rate of change of biological solids concentration in the reactor

F = rate of inflow of growth medium or wastewater

Figure 1 - Flow diagram, completely-mixed reactor employing cell recycle. See text for definition of symbols.


INFLUENT
F
X_i , S_i
REACTOR
X,S
RECYCLE, αF
CX, S_r
(1+α)F
X , S
CLARIFIER
F
X_e , S
EFFLUENT

x_i = biological solids concentration in the inflowing waste

α = recirculation ratio, i.e., ratio of the rate of underflow from the clarifier to the rate of inflowing waste

c = concentration factor, i.e., the ratio of the biological solids concentration in the clarifier underflow to the biological solids concentration in the reactor

μ = logarithmic growth rate constant for the biological population (natural logarithm)

x = biological solids concentration in the reactor.

As in the case of once-through systems, the overall dilution rate, D, for the entire system, i.e., reactor and clarifier, is F/V. However, the dilution rate for the reactor, D_1, is increased by the factor $(1 + \alpha)$, i.e, $D_1 = (1 + \alpha)F/V$ (see last term in materials balance, Equation 1). For the case wherein there are essentially no biological solids in the inflow ($x_i = 0$), Equation 1 may be written as follows:

$$\frac{dx}{dt} = D\alpha cx + \mu x - (1 + \alpha)Dx \qquad (2)$$

Under steady state conditions, $\frac{dx}{dt} = 0$, and the equation may be rearranged as follows:

$$\mu = D(1 + \alpha - \alpha c) \qquad (3)$$

or $$\mu = DA \text{ where } A = (1 + \alpha - \alpha c) \qquad (3a)$$

It should be noted that in systems employing cell recycle, μ is not equal to D for the reactor, i.e., D_1, as was the case for a once-through system (1). Substituting the value of D in terms of D_1 (i.e., $D = D_1/(1 + \alpha)$ in Equation 3, it may be seen that μ is smaller than D_1; it is reduced by the factor $\alpha c/(1 + \alpha)$, i.e.,

$$\mu = \left[1 - \frac{\alpha c}{1 + \alpha}\right] D_1 \tag{4}$$

The growth rate, in a strict sense, is not wholly controlled hydraulically; it is subject to the effect of the recycle concentration factor, c, a nonhydraulic factor.

The rate of change in the substrate concentration, $\frac{dS}{dt}$, may be expressed as follows:

increase = inflow + recycle - outflow - consumption for growth

$$V \left(\frac{dS}{dt}\right) = FS_i + \alpha FS_r - (1 + \alpha)FS - V \frac{\mu x}{Y} \tag{5}$$

where Y is the cell yield defined as the ratio of the concentration of cells formed to the concentration of substrate consumed (weight/volume basis) and S_r is the concentration of substrate in the recycle.

When there is essentially no substrate in the recycle, i.e., $S_r \ll S_i$, Equation 5 may be written as follows:

$$\frac{dS}{dt} = DS_i - (1 + \alpha)DS - \mu \frac{x}{Y} \tag{6}$$

and in the steady state the concentration of cells in the reactor, $\bar{x}$, may be expressed in accordance with the following equation:

$$\bar{x} = \frac{DY \left[S_i - (1 + \alpha)S\right]}{\mu} \tag{7}$$

Since, from Equation 3

$$\mu = D(1 + \alpha - \alpha c)$$

or, for convenience

$$\mu = DA \tag{3a}$$

where $A = (1 + \alpha - \alpha c)$

the value of $\bar{x}$ may be expressed as follows:

$$\bar{x} = Y\left[\frac{S_i - (1 + \alpha)\bar{S}}{1 + \alpha - \alpha c}\right] = \frac{Y}{A}\left(S_i - (1 + \alpha)\bar{S}\right) \tag{8}$$

In this equation, $\bar{S}$ is the steady state concentration of substrate in the reactor.

When there is sufficient substrate carried over into the recycle to warrant its consideration, the steady state equation for $\bar{x}$ may be given as follows:

$$\bar{x} = \frac{Y(S_i - \bar{S})}{(1 + \alpha - \alpha c)} = \frac{Y}{A}(S_i - \bar{S}) \tag{9}$$

where S_r is assumed to be equal to $\bar{S}$.

The steady state substrate concentration, $\bar{S}$, may be obtained by combining Equation 3 with the Monod equation, which expresses the relation between growth rate and substrate concentration:

$$\mu = \frac{\mu_m S}{k_s + S} \tag{10}$$

where μ_m is the maximum logarithmic growth rate of the cells, i.e., nonsubstrate-limited growth, and k_s is the saturation constant, numerically equal to the substrate concentration at which $\mu = 1/2\ \mu_m$.

Substituting for μ in Equation 3, the steady state concentration of $\bar{S}$ is given by the following equations:

$$\bar{S} = \frac{k_s D(1 + \alpha - \alpha c)}{\mu_m - D(1 + \alpha - \alpha c)} \tag{11}$$

or

$$\bar{S} = \frac{k_s AD}{\mu_m - AD} \tag{12}$$

By comparison of the steady state equations for $\bar{x}$ and $\bar{S}$ for systems in which cell recycle is practiced with the steady state equations for these parameters for once-through systems (1), it may be discerned that cell (or sludge) recycle should allow the system to operate successfully (i.e., provide good substrate removal) at lower detention times (higher dilution rates).

For once-through systems the cell output (mg/l/hr) was defined as $D\bar{x}$, and this product was equivalent to the rate of production of waste sludge or cells. In Herbert's theoretical analysis for systems employing cell feedback (3), separation of the reactor mixed liquor was accomplished by continuous centrifugation and the cell output for the overall system was defined as the product of D and the concentration of cells (x_2) in the centrifugate, i.e., in the effluent supernatant. It was further shown that x_2 could be expressed as follows:

$$x_2 = \bar{x}(1 + \alpha - \alpha c) = \bar{x}A \qquad (13)$$

Therefore,

$$\text{Cell output} = D\bar{x}A \qquad (14)$$

In the present study, centrifugation was not employed because activated sludge processes, under present modes of operation, depend upon flocculation and quiescent settling to effect separation of the reactor mixed liquor. If settling is essentially one hundred per cent efficient, one may calculate an equivalent cell output (or waste sludge rate) using Equation 14 and by multiplying by the volume of the reactor may arrive at the sludge wasting rate in mg/hr.

MATERIALS AND METHODS

The general experimental procedures and analyses employed in the present study were the same as those previously reported for work conducted at a feeding level of 1000 mg/l glucose (1). For studies at a loading level of 3000 mg/l glucose (once-through reactor), the concentration of mineral salts was increased three-fold, whereas for studies at a feeding level of 1000 mg/l glucose (cell recycle reactor) the medium was identical to that previously used (1).

Heterogeneous microbial populations were developed from an initial sewage seed obtained from the municipal sewage treatment plant at Stillwater, Oklahoma. The reactor was the one previously employed (see Figure 2). However, for studies on the cell recycle system, additional aeration (return sludge aerator - see Figure 3) was introduced. Underflow from the clarifier was periodically channelled to the return sludge aerator, diluted to the desired consistency for recycle, and pumped to the aeration tank at the desired flow rate. The remaining underflow comprised the waste sludge. For all studies (four at D = 1/7.5, 1/5, 1/3.75, and 1/2.5 hour^{-1}) the recirculation factor, α, was maintained at 0.25 and the sludge concentration factor, c, was held as closely as possible to 1.5.

The reactor was shown to be completely mixed by the close agreement observed between experimental and theoretical dilute-in curves, and by the fact that biological solids concentrations in the reactor and reactor effluent were essentially the same. All studies were conducted at a temperature of $23^{\circ}\text{C} \pm 0.5$;

Figure 2 - Side view of completely-mixed continuous flow reactor employed in laboratory studies of "once-through" systems.

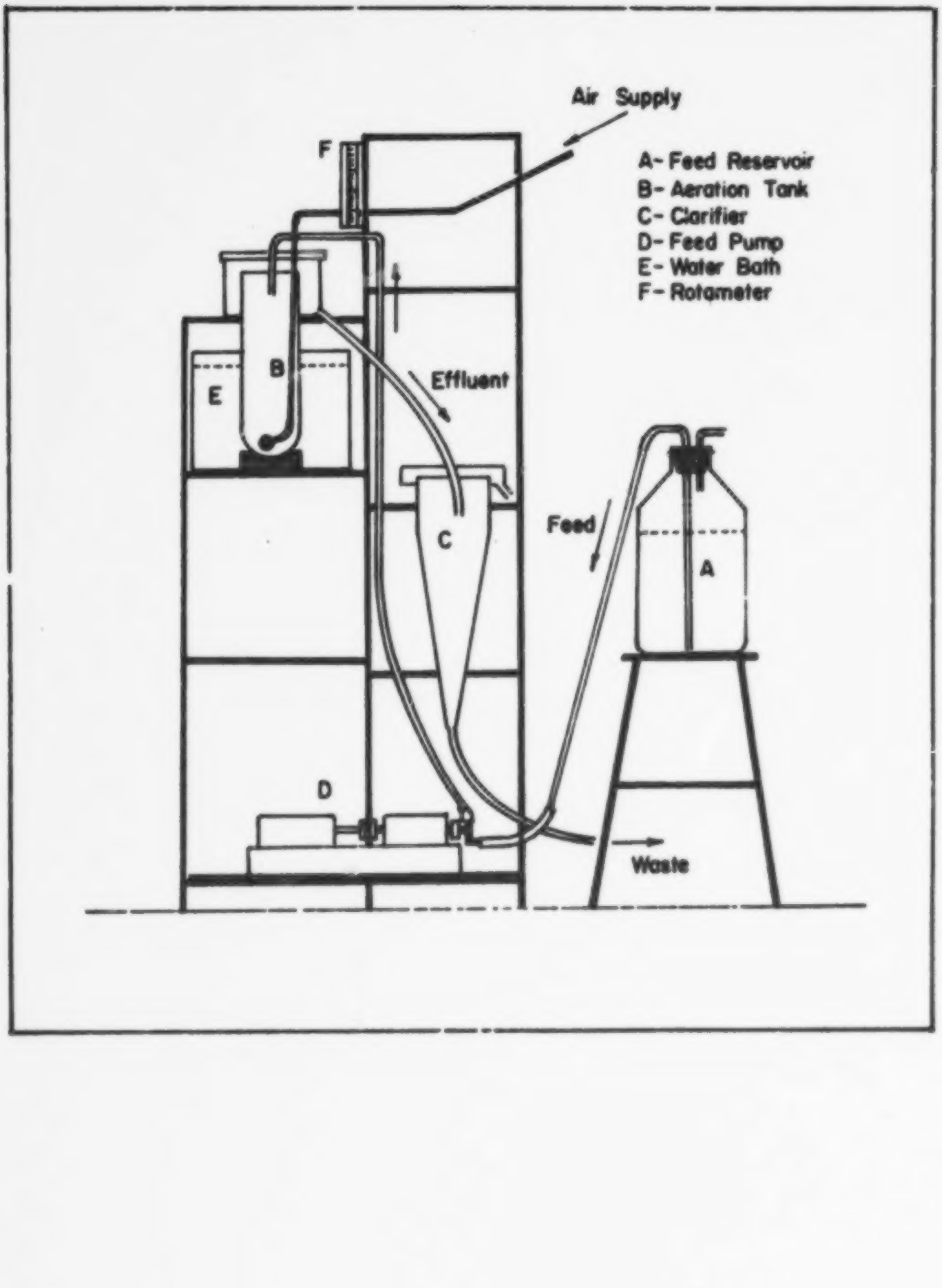
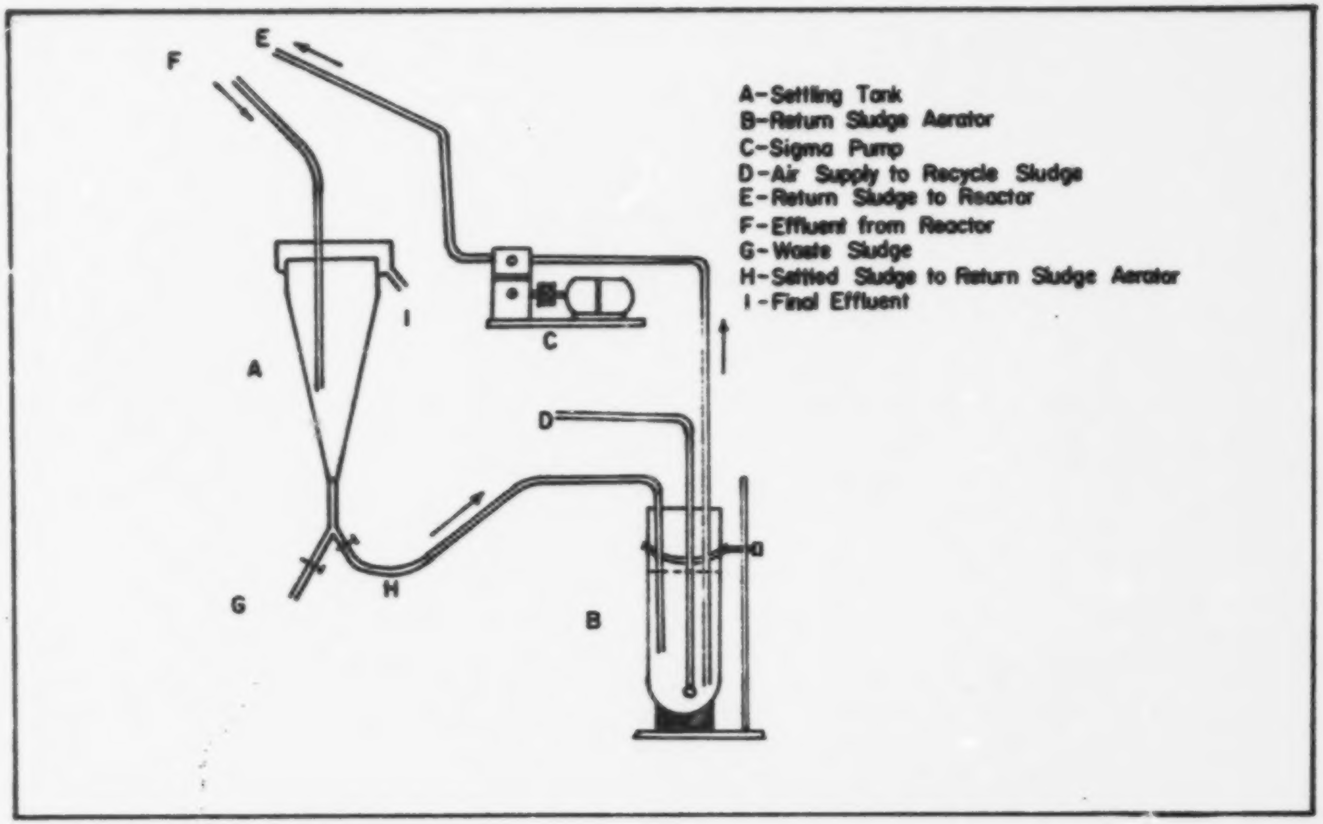

Air Supply
F
A- Feed Reservoir
B- Aeration Tank
C- Clarifier
D- Feed Pump
E- Water Bath
F- Rotameter
B
E
Effluent
Feed
C
A
D
Waste

Figure 3 - Side view of sludge recycle system added to pilot plant shown in Figure 2 for studies employing cell feedback to reactor.


F
E
A-Settling Tank
B-Return Sludge Aerator
C-Sigma Pump
D-Air Supply to Recycle Sludge
E-Return Sludge to Reactor
F-Effluent from Reactor
G-Waste Sludge
H-Settled Sludge to Return Sludge Aerator
I-Final Effluent
I
C
A
D
G
H
B

pH was maintained at 6.8 ± 0.2, and dissolved oxygen concentration in the aerator (measured electrometrically) was maintained above ninety per cent of the saturation concentration. The biological solids concentrations in the reactor (or its effluent) and in the return sludge were measured using the membrane filter technique. The protein and carbohydrate content of the sludge were also assessed (4). The membrane filtrate was analyzed for its chemical oxygen demand (COD)(5) and for carbohydrate content using the anthrone method (4). For the studies employing cell recycle, the COD of the clarifier supernatant was also measured. Since there was, in this study as in the previous work, some variation in "steady state" levels for biological solids and substrate, the mean levels of all parameters were estimated statistically.

At various times during operation at each specific dilution rate, cells were harvested for batch experiments to determine μ_m and k_s using the same experimental protocol employed in the previous study (1). Cell yields for the batch studies (Y_B) were also measured for comparison with the cell yields determined from the continuous flow data (Y_C). The "unit activity" of the harvested sludge, with respect to O_2 uptake, was measured using the Warburg apparatus (mg/O_2/gm sludge /hr, under endogenous conditions).

RESULTS

Systems of the "once-through" type (S_i = 3000 mg/l glucose)

As was the case for the research reported previously (1) using 1000 mg/l glucose, the conduct of the present work required massive experimental effort and analyses of many samples for experiments conducted at the various dilution rates employed. In the interest of brevity, the average values for all experimental parameters are presented in table form, and results typical of individual experimental runs are presented graphically.

Figure 4 shows the variation in the experimental parameters during "steady state" operation at a dilution rate of 1/3 hr^{-1}. The variations observed are typical of those which occurred at the other dilution rates studied. As was the case for the studies at S_i of 1000 mg/l glucose (1), the biological solids concentration exhibited the greatest fluctuation (from slightly over 2000 mg/l to approximately 1500 mg/l). These changes in biological solids concentration were not attributable to random errors in sampling or in analytical techniques, since many samples taken over short time intervals, a procedure which was employed in the previous study, did not show such variation. There was also considerable variation in the concentration of filtrate COD. The average steady state values for biological solids concentration, $\bar{x}$, effluent COD, $\bar{S}$, influent COD, S_i, and effluent carbohydrate concentration, $\bar{S}_c$, during operation at this dilution rate are given in Table I along with average values obtained from studies similar to those shown in Figure 4 for all dilution rates studied in this series of experiments. Also given are the coefficients of variance for biological solids and effluent substrate concentration (columns 6, 7, and 8). The

Figure 4 - "Steady-state" parameters at $D = 1/3\ hr^{-1}$ (S_i = 3000 mg/l glucose; "once-through" system).

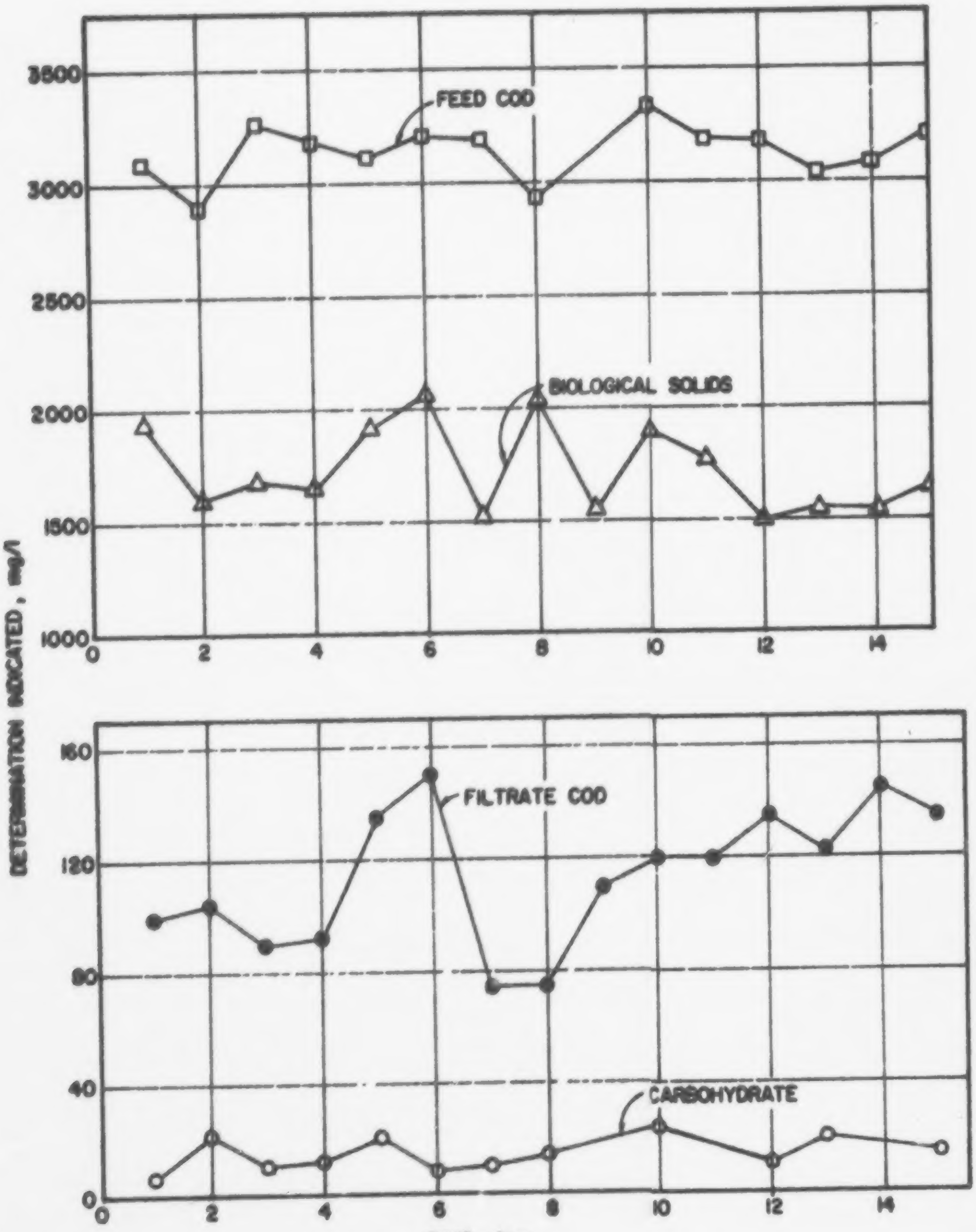

FEED COD
BIOLOGICAL SOLIDS
FILTRATE COD
CARBOHYDRATE
3500
3000
2500
2000
1500
1000
160
120
80
40
0
0
2
4
6
8
10
12
14
DETERMINATION INDICATED, mg/l
TIME, days

TABLE I

STATISTICAL ESTIMATION OF THE "STEADY-STATE" PARAMETERS AT DIFFERENT DILUTION RATES

(S_i = 3000 mg/l Glucose; Without Cell Recycle)

Dilution Rate, hr^{-1} (1)		$\bar{x}$[a] mg/l (2)	$\bar{S}$[b] mg/l (3)	S_i[c] mg/l (4)	S_c[d] mg/l (5)	Coefficient of Variance, % $\bar{x}$ (6)	$\bar{S}$ (7)	$\bar{S}_c$ (8)	$\frac{D(S_i - \bar{S})}{\bar{x}}$ mg COD/ mg SS/hr (9)	Loading Factor lb COD/ lb SS/day (10)	COD Removal Efficiency % (11)
1/24	0.042	1589	221	3048	43	32.2	71.3	144.0	0.07	4.80	92.6
1/18	0.056	2010	87	3121	9	23.4	18.4	23.2	0.08	5.17	97.0
1/12	0.083	1993	112	3086	11	19.7	54.5	32.8	0.12	7.74	96.5
1/6	0.167	1917	120	3147	17	20.2	28.4	69.5	0.26	16.4	96.2
1/3	0.333	1731	113	3139	17	11.7	20.3	45.4	0.58	36.3	96.4
1/2	0.500	1787	224	3163	31	13.6	23.7	38.9	0.82	53.1	92.9
1/1.5	0.667	676	1569	2950	1225	46.4	36.5	74.5	1.36	175	46.8
1/1	1.000	122	2745	3080	2535	97.1	10.1	7.1	2.75	1515	10.9

a. $\bar{x}$ = mean biological solids

b. $\bar{S}$ = mean effluent COD

c. $\bar{S}_i$ = mean influent COD

d. $\bar{S}_c$ = mean effluent carbohydrate specific substrate removal rate, S_R, shown in column 9, was calculated in accordance with the following equation:

$$S_R = \frac{D\left(S_i - S\right)}{X} \qquad (15)$$

Also shown are the loading factors and COD removal efficiencies during operation at each dilution rate.

At various times during operation at each dilution rate, cells were harvested from the reactor effluent and used in batch experiments to determine the nature of the relation between μ and substrate concentration, S, in precisely the same manner as was done during the previous study (see Figures 2 and 3 of reference 1). The data were tested for fit in accordance with the relationship proposed by Monod (6), Moser (7), and Teissier, as described by Schulze (8)(9). It was found that the Monod equation provided the best approximation of the μ vs. S curves drawn through the observed data. This result was in accord with that of the previous study using cells harvested from the continuous flow reactor operating at an S_i of 1000 mg/l glucose.

In Table II the values of μ obtained for various substrate (glucose) concentrations employing cells harvested from the reactor effluent at the various dilution rates are given along with the μ_m and k_s values determined from plots of μ vs. S, $1/\mu$ vs. 1/S, and the average of the values obtained by both methods. The average yield coefficients obtained in the batch experiments (column 14) are compared with those obtained using the continuous flow data (column 15).

TABLE II

GROWTH PARAMETERS OBTAINED FROM BATCH EXPERIMENTS ACCORDING TO MONOD'S EQUATION

(S_i - 3000 mg/l Glucose; Without Cell Recycle)

Dilution Rate, hr^{-1}		Specific Growth Rate, hr^{-1} for Concentration of Substrate, mg/l						$\mu_{m_1}{}^{a}$	$k_{s_1}{}^{a}$	$\mu_{m_2}{}^{b}$	$k_{s_2}{}^{b}$	$\mu_{m}{}^{c}$	$k_{s}{}^{c}$	Yield Coeff.	
		50	100	200	300	500	800	hr^{-1}	mg/l	hr^{-1}	mg/l	hr^{-1}	mg/l	Y_B batch	Y_C cont.
(1)		(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)
1/24	.042	.254	.257	.363	.393	.432	.364	0.43	52	0.44	37	0.43	45	0.61	0.56
1/18	.056	.169	.203	.230	.295	.359	.418	0.38	90	0.40	93	0.39	92	0.41	0.66
1/12	.083	.228	.375	.472	.500	.600	.615	0.60	65	0.72	105	0.66	85	0.47	0.67
1/6	.167	.200	.241	.306	.363	.446	.488	0.53	115	0.50	108	0.52	113	0.45	0.63
1/3	.333	.336	.386	.393	.400	.459	.458	0.46	22	0.45	18	0.46	20	0.56	0.57
1/2	.500	.503	.528	.608	.605	.620	-	0.64	30	0.63	13	0.63	22	0.50	0.61
1/1.5	.667	.365	.482	.530	.552	.500	-	0.55	30	0.63	58	0.59	29	0.44	0.49
1/1	1.000	.250	.312	.376	.410	.416	.410	0.41	50	0.48	52	0.45	51	0.36	0.37

a. μ_{m_1} and k_{s_1} obtained from the graph of μ vs. S.

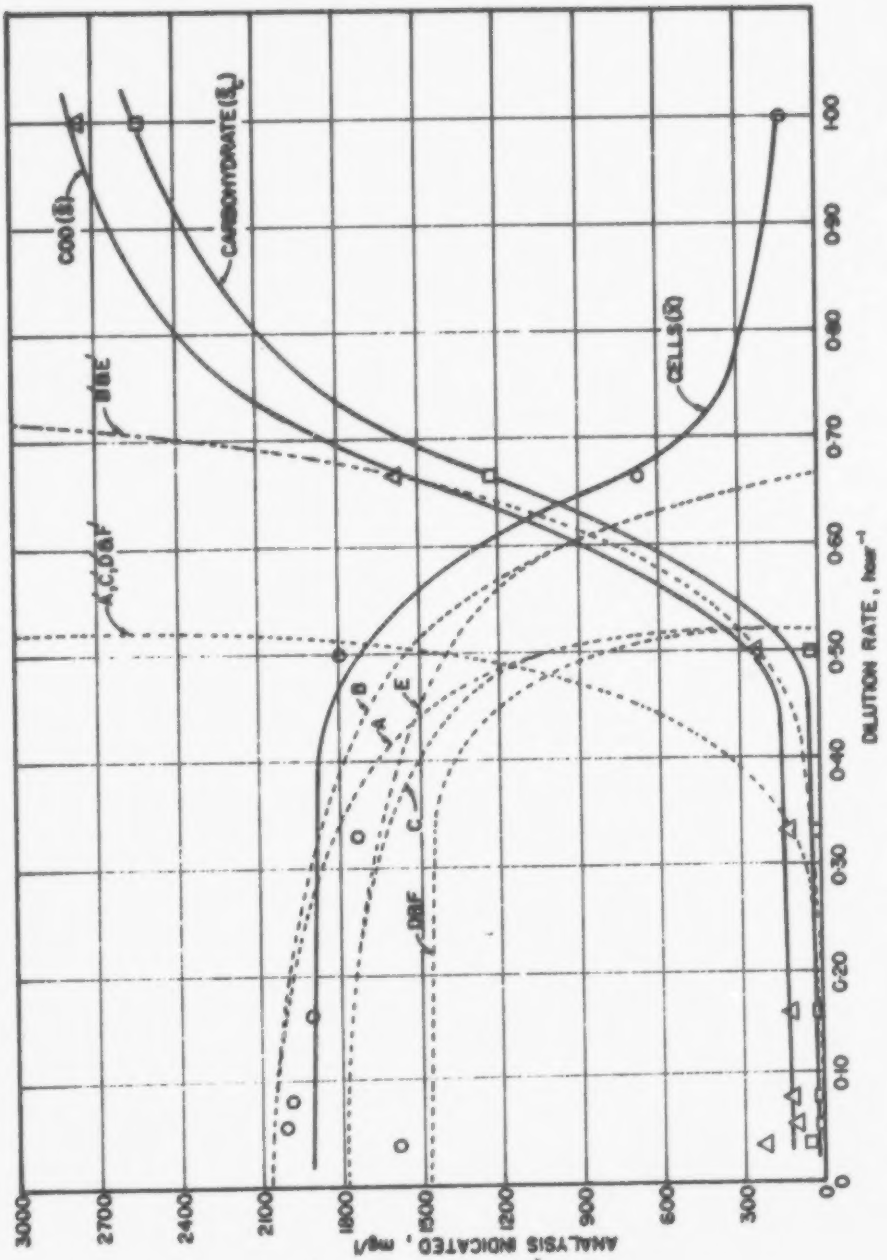
b. μ_{m_2} and k_{s_2} obtained from the graph of $1/\mu$ vs. 1/S.

c. μ_m and k_s are the mean values of μ_{m_1} and μ_{m_2}, and k_{s_1} and k_{s_2}.

The average values (from Table I) for steady state solids concentration, effluent COD and carbohydrate are plotted in Figure 5 (solid line curves). It is seen that the system remained fairly stable up to a dilution rate of 0.5 hr^{-1} (detention time two hours). At higher dilution rates, the cells began to dilute out, and effluent substrate rose sharply. The dotted line curves represent the theoretical dilute-out curves calculated in accordance with the theoretical kinetic formulation for once-through systems (see Equations 8 and 9 of the previous report (1)) using the various μ_m, k_s, and Y values obtained experimentally during this study. It may be seen that the solids concentration curve labeled B and the substrate curves labeled B' and E' provide the best approximation of the observed data. Curves B and B' were generated using the individual values of μ_m, k_s, and cell yield (Y_C) obtained at each dilution rate, whereas curves E and E' were calculated from the same values for μ_m and k_s as used for the B curves and the yield values (Y_B) obtained in the batch studies accompanying each dilution rate. The levels of biological solids and substrate concentrations using the "constants" employed for the B curves provide fairly good approximations of the observed kinetic behavior at dilution rates up to the range 0.6 - 0.7 hr^{-1}. The computed or theoretical curves, however, do not account for the tailing-off of both biological solids and substrate concentration at the higher dilution rates, i.e., at the dilution rates at which the cells should have been

Figure 5 - Dilute-out curves for solids and substrate (S_i = 3000 mg/l glucose; "once-through" system). Solid lines show observed data. Curves A to F and A' to F' show calculated values for biological solids and substrate based on the Monod equation and using the following values for the constants μ_m, k_s, and Y:

Curves A and A', mean values of μ_m and k_s from batch data and Y_C values for each dilution rate.
Curves B and B', individual values of μ_m, k_s, and Y_C for each dilution rate.
Curves C and C', mean values of μ_m, k_s, and Y_C.
Curves D and D', mean values of μ_m, k_s, and Y_B.
Curves E and E', individual values of μ_m, k_s, and Y_B from batch studies at each dilution rate.
Curves F and F', mean values of μ_m and k_s from batch data and Y_B values for each dilution rate.


COD (S)
CARBOHYDRATE (S_c)
CELLS (X)
B & E
A, C, D & F
B
A
E
C
D & F
DILUTION RATE, hour^{-1}
0
0·10
0·20
0·30
0·40
0·50
0·60
0·70
0·80
0·90
1·00
ANALYSIS INDICATED, mg/l
0
300
600
900
1200
1500
1800
2100
2400
2700
3000

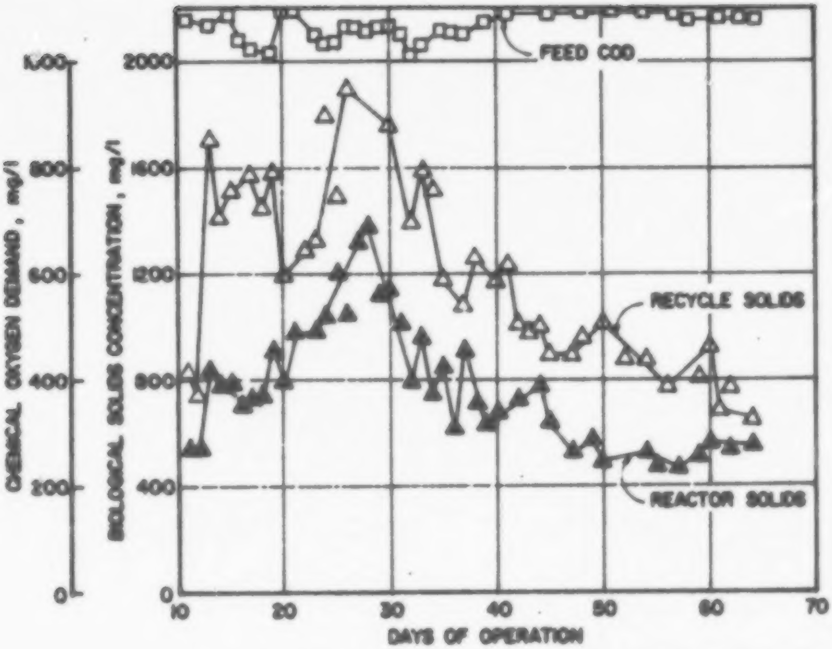
totally washed out of the reactor. This tailing-off effect was noted in the previous studies (1), and has been found to occur also in studies employing pure cultures of microorganisms (2).

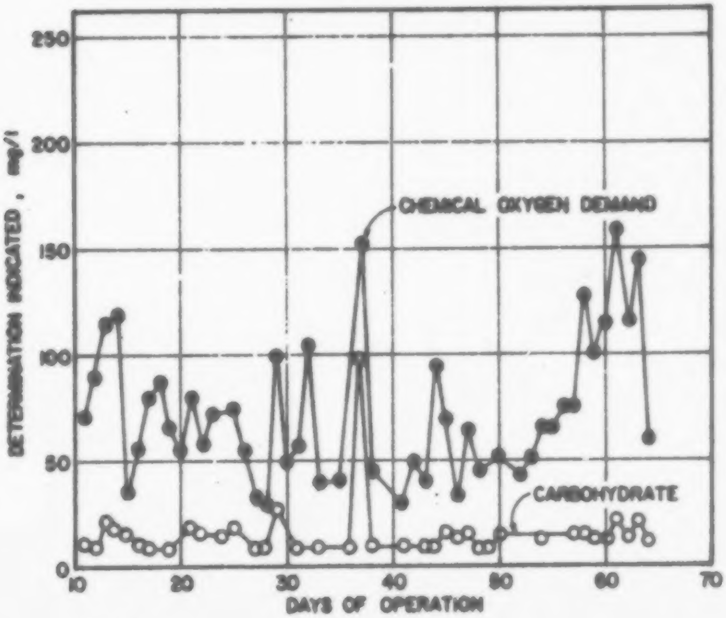
Systems employing cell recycle (S_i = 1000 mg/l glucose)

A series of continuous culture experiments was made, employing cell recycle at a volumetric flow rate of twenty-five per cent of the synthetic waste flow ($\alpha = 0.25$) with the concentration of recycle solids at one hundred fifty per cent of the reactor solids level ($c = 1.5$).

Figure 6 shows the variation in the experimental parameters during "steady state" operation at a dilution rate of 1/3 hr^{-1}. It will be recalled that in order to use the continuous flow steady state theory which has been developed and to employ the concentration factor, c, for cell recycle, it is necessary to maintain c as constant as possible. Since with heterogeneous populations there is ample evidence that biological solids concentration fluctuates rather widely about an average "steady state" value (see reference 1 and results of this study), it was necessary to vary the concentration of recycled solids in order to maintain c at a level approaching a constant value of 1.5. This was at times difficult to do (note first twenty days of operation, Figure 6). In the activated sludge process, separation of the mixed liquor solids and effluent is brought about by autoflocculation and settling of the solids. Any other method cannot as yet be economically justified. Therefore, the

Figure 6 - "Steady-state" parameters at $D = 1/3\ hr^{-1}$ (S_i = 1000 mg/l glucose; sludge recycle with α = 0.25 and c = 1.5).


FEED COD
RECYCLE SOLIDS
REACTOR SOLIDS
CHEMICAL OXYGEN DEMAND, mg/l
BIOLOGICAL SOLIDS CONCENTRATION, mg/l
DAYS OF OPERATION
0
200
400
600
800
1000
0
400
800
1200
1600
2000
10
20
30
40
50
60
70


CHEMICAL OXYGEN DEMAND
CARBOHYDRATE
DETERMINATION INDICATED, mg/l
DAYS OF OPERATION
0
50
100
150
200
250
10
20
30
40
50
60
70

efficiency of the clarifier or settling tank is subject to the vagaries of microbial auto-flocculation and subsidence, and is by no means constant. This was the primary reason why a separate return sludge aeration tank was employed in these studies rather than direct recycling of a portion of clarifier underflow.

In general, it may be discerned that after the first twenty days of operation, c was maintained fairly constant, i.e., the recycle solids concentration approximated one hundred fifty per cent of the reactor solids concentration. It should also be pointed out that while the effluent substrate values plotted (COD and carbohydrate) are based on determinations run on filtrate rather than clarifier supernatant, spot checks on the clarifier supernatant indicated that for over ninety per cent of the total experimental period during all experiments employing cell recycle, the supernatant values were only 25-30 mg/l higher than the filtrate values. This finding attests to the rather good settleability of these systems. However, there were times when settling was not a very efficient means of separating the mixed liquor. The filtrate values, rather than the supernatant values, were employed in all calculations and data analyses because many more such values were available for the statistical analyses needed to establish approximate "steady state" concentrations and because of the desirability of making direct comparison of effluent levels with those of once-through systems operated at comparable dilution ratios.

2 OF 4

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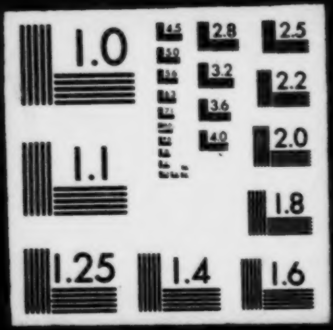


Supernatant values could not be used for the once-through systems, because this mode of operation leads, in general, to dispersed microbial populations which do not flocculate and settle in the clarifier.

Although not a subject for detailed study in the present investigation, it is interesting to observe the beneficial effect of cell recycle on the flocculating and settling characteristics of the microbial population. Explanation of this effect awaits further investigations, but it may be linked to the fact that under recycle conditions the cells undergo a period of endogenous respiration or metabolic dormancy in the clarifier (and in the present case, in the return sludge aerator) which may enhance flocculation tendencies. Also, the average age of the cells in these systems is higher than in once-through systems. Also, we have observed that the protozoan population is generally higher in systems employing cell recycle. While these observations have been made repeatedly over a period of years of study on the kinetics and mechanisms of activated sludge, and while they do bear on some of the prevalent theories concerning microbial flocculation, it is presently not possible to provide a unifying theoretical explanation of the phenomenon. These observations do appear worthy of note, however, because of the present importance of the subject and the dependence on flocculation and quiescent settling for the operation of activated sludge processes.

Average values for biological solids and substrate concentrations computed for the data at $D = 1/3 \text{ hr}^{-1}$ (shown in Figure 6) are given in Table III along with the average values obtained from similar studies at the other dilution rates examined. The coefficients of variance for the major parameters (columns 8, 9, 10, and 11), and the specific substrate utilization rates, loading factors, and system efficiencies are also shown.

The average results of batch experiments which were made to determine the kinetic "constants" using cells harvested at various times during operation at each dilution rate, are given in Table IV. These data were also tested for fit in accordance with the relationships between μ and S proposed by Monod (6), Moser (7), and Teissier (8)(9). While in some cases there was enough scatter in the data to make it difficult to decide which model best described the relationship between μ and S (especially true of the data at $D = 1/3$), the results, in general, support the applicability of the Monod relation. Also shown in Table IV are the cell yield values obtained under both batch and continuous flow conditions. The equation employed for calculation of Y_C is given in a footnote.

The average values (from Table III) for the steady state solids concentration, effluent COD, and carbohydrate at each dilution rate are plotted in Figure 7 (solid line curves). It is seen that these systems followed a general dilute-out pattern similar to that observed for the once-through reactor systems. The dotted lines represent the theoretical dilute-

TABLE III

STATISTICAL ESTIMATION OF THE "STEADY-STATE" PARAMETERS AT DIFFERENT DILUTION RATES
$\left[S_i = 1000\ \text{mg/l Glucose; With Cell Recycle},\ \alpha = 0.25,\ c = 1.50\ \text{(nominally)}\right]$

Dilution Rate, hr^{-1} (1)		Mean Reactor Residence time, hr. (2)	$\bar{x}^a$ mg/l (3)	$c\bar{x}^b$ mg/l (4)	$\bar{S}^c$ mg/l (5)	$\bar{S}_i^d$ mg/l (6)	$\bar{S}_c^e$ mg/l (7)	Coefficient of Variance, % $\bar{x}$ (8)	$c\bar{x}$ (9)	$\bar{S}$ (10)	$\bar{S}_c$ (11)	$\frac{D_1(S_i-\bar{S})}{\bar{x}}$ mg COD/ mg SS/hr (12)	Loading Factor lb COD/ lb SS/day (13)	COD Removal Efficiency % (14)
1/7.5	.133	6	837	1295	60	1040	6	21.9	27.8	36.0	87.5	0.195	9.94	94.2
1/5	.200	4	797	1236	87	1060	15	32.4	44.7	36.0	56.8	0.305	16.0	91.7
1/3.75	.267	3	753	1121	79	1067	17	31.2	48.8	56.1	117.5	0.438	22.6	92.6
1/2.5	.400	2	500	861	277	1065	156	39.3	48.0	66.7	111.5	0.788	51.1	74.0

a. $\bar{x}$ = mean biological solids

b. $c\bar{x}$ = mean biological solids in the recycle

c. $\bar{S}$ = mean effluent COD

d. $\bar{S}_i$ = mean influent COD

e. $\bar{S}_c$ = mean effluent carbohydrate

TABLE IV

GROWTH PARAMETERS OBTAINED FROM BATCH EXPERIMENTS ACCORDING TO MONOD'S EQUATION
(S_1 = 1000 mg/l; with cell recycle)

Dilution Rate, hr^{-1}	Mean Reactor Residence Time, hr	Specific Growth Rate, hr^{-1} for Concentration of Substrate, mg/l						μ_{m_1}[a] hr^{-1}	k_{s_1}[a] mg/l	μ_{m_2}[b] hr^{-1}	k_{s_2}[b] mg/l	μ_m[c] hr^{-1}	k_s[c] mg/l	Yield Coeff. Y_B	Yield Coeff. Y_C[d]
		50	100	200	300	500	800								
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)
1/7.5 .133	6	.195	.262	.312	.289	.337	.387	0.39	60	0.40	58	0.40	59	0.38	0.76
1/5 .200	4	.111	.354	.530	.532	.648	.560	0.67	90	0.74	111	0.70	100	0.58	0.73
1/3.75 .267	3	.221	.146	.216	.306	.398	.433	0.30	23	0.31	23	0.31	23	0.59	0.68
1/2.5 .400	2	.202	.235	.311	.421	.470	.407	0.46	85	0.46	85	0.46	85	0.44	0.61

a. μ_{m_1} and k_{s_1} obtained from the graph μ vs. S.

b. μ_{m_2} and k_{s_2} obtained from the graph $1/\mu$ vs. 1/S.

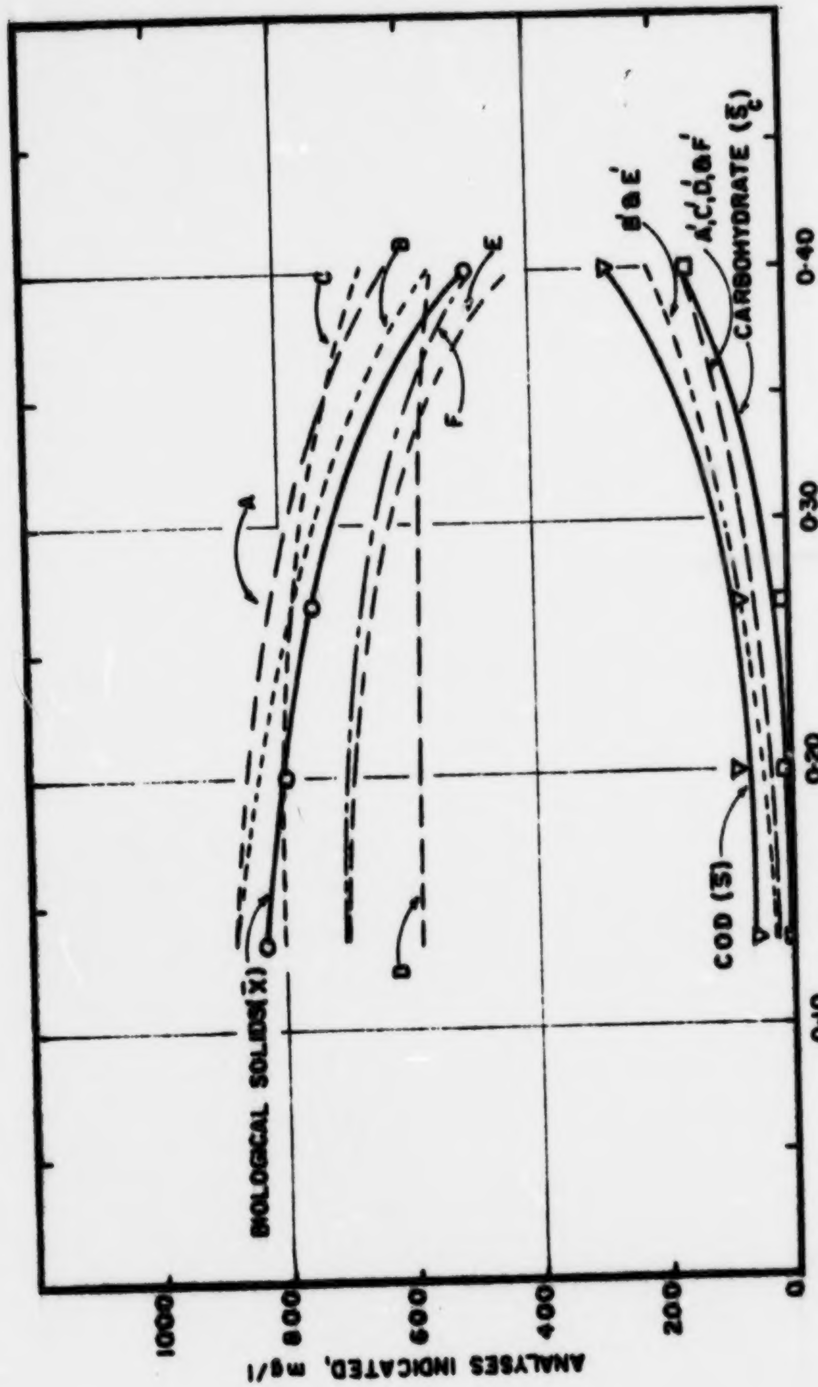
c. μ_m and k_s are the mean values of μ_{m_1} and μ_{m_2}, and k_{s_1} and k_{s_2}.

d. Y_C obtained from the equation:

$$Y_C = \frac{(1 + \alpha - \alpha c)\bar{x}}{\left[S_1 - (1 + \alpha)\bar{S}\right]}$$

Figure 7 - Dilute-out curves for solids and substrate (S_i = 1000 mg/l glucose; sludge recycle with α = 0.25 and c = 1.5). Solid lines show observed data. Curves A to F and A' to F' show calculated values for biological solids and substrate based on the Monod equation and using the following values for the constants μ_m, k_s, and Y:

Curves A and A', mean values of μ_m and k_s from batch data and Y_C values for each dilution rate.
Curves B and B', individual values of μ_m, k_s, and Y_C for each dilution rate.
Curves C and C', mean values of μ_m, k_s, and Y_C.
Curves D and D', mean values of μ_m, k_s, and Y_B.
Curves E and E', individual values of μ_m, k_s, and Y_B from batch studies at each dilution rate.
Curves F and F', mean values of μ_m and k_s from batch data and Y_B values for each dilution rate.


ANALYSES INDICATED, mg/l
1000
800
600
400
200
0
DILUTION RATE, D, hours^{-1}
0·10
0·20
0·30
0·40
BIOLOGICAL SOLIDS($\bar{X}$)
A
B
C
D
E
F
COD ($\bar{S}$)
B' & E'
A',C',D',& F'
CARBOHYDRATE ($\bar{S}_c$)

out curves calculated in accordance with Equations 7 and 11, using the various μ_m, k_s, and Y values obtained experimentally during the investigation, i.e., the various values of these parameters given in Table IV. It is seen that curves A and B yield the best approximation of the observed solids dilute-out curve, i.e., either the mean of all of the data or the individual values of μ_m and k_s for each dilution rate coupled with the individual Y_C values for each dilution rate provide a satisfactory fit of the observed "steady state" data. The continuous flow studies were not carried forward to dilution rates approaching total dilute-out, since it was obvious that dilute-out was well under way at the highest D (0.5 hr^{-1}) employed.

Sludge composition and unit activity of the cells

Throughout the previous continuous flow study at an S_i of 1000 mg/l glucose (1) and the present study, the protein and carbohydrate contents of the sludge were determined at each sample time for which biological solids, filtrate COD, and filtrate carbohydrate were assayed. Also, in the present study, O_2 uptake was measured (Warburg respirometer) during the batch studies performed to determine the kinetic constants μ_m and k_s. The average values for all sludge composition and unit activity determinations at each dilution rate are shown in Table V. It is seen that for the once-through systems, the protein content decreased, and the carbohydrate content increased as the dilution rate was increased. This trend was not apparent for the system employing cell recycle.

TABLE V

COMPOSITION AND UNIT ACTIVITY OF SLUDGE AT DIFFERENT DILUTION RATES

Dilution Rate, hr^{-1}	S_i = 1000 mg/l Glucose					S_i = 3000 mg/l Glucose		
	Without Cell Recycle		With Cell Recycle			Without Cell Recycle		
	Protein %	Carbo. %	Protein %	Carbo. %	Unit Act.[a]	Protein %	Carbo. %	Unit Act.[a]
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)
1/24 .042	58	13	-	-	-	52	11	6.3
1/18 .056	60	10	-	-	-	67	13	8.1
1/12 .083	63	11	-	-	-	53	12	10.6
1/7.5 .133	-	-	60	28	2.1	-	-	-
1/6 .167	62	22	-	-	-	61	23	7.4
1/5 .200	-	-	54	32	1.1	-	-	-
1/4 .250	59	25	-	-	-	-	-	-
1/3.75 .267	-	-	61	34	1.4	-	-	-
1/3 .333	42	32	-	-	-	50	29	5.8
1/2.5 .400	-	-	53	33	2.5	-	-	-
1/2 .500	41	31	-	-	-	42	30	1.0
1/1.5 .667	39	36	-	-	-	42	31	0.5
1/1 1.000	-	-	-	-	-	38	35	0.4

[a] Unit activity of sludge was measured and expressed as mg of oxygen consumed per hour per gram of washed sludge.

The unit activity for cells harvested from the once-through system varied from 6.3 to 10.6 mg O_2/gm cells, with no discernible trend, for dilution rates of 1/3 hr^{-1} and below, but dropped off rather sharply at the higher flow rates. The unit activity of the cells harvested from the systems employing cell recycle was in general lower than for cells taken from the once-through systems.

Specific substrate removal rate

The specific substrate removal rates at the various dilution rates for the feed concentrations used in the previous study (1) and in the present experimentation are plotted in Figure 8. In general, the specific substrate removal rate appears to increase linearly with increasing dilution rate.

Effect of dilution rate on values of μ_m determined in batch studies

In the previous work (1) it was noted that, in general, μ_m values determined in batch studies using cells harvested at various dilution rates increased with increasing dilution rates (i.e., decreasing reactor detention time). In Figure 9 the average μ_m values are plotted vs. dilution rate, D, for all data of the previous and the present studies. It is seen that there is a considerable amount of scatter in the data, but the line of best fit (by least squares) indicates an increase in μ_m at the higher dilution rates.

Figure 8 - Effect of dilution rate on the specific substrate removal rate.

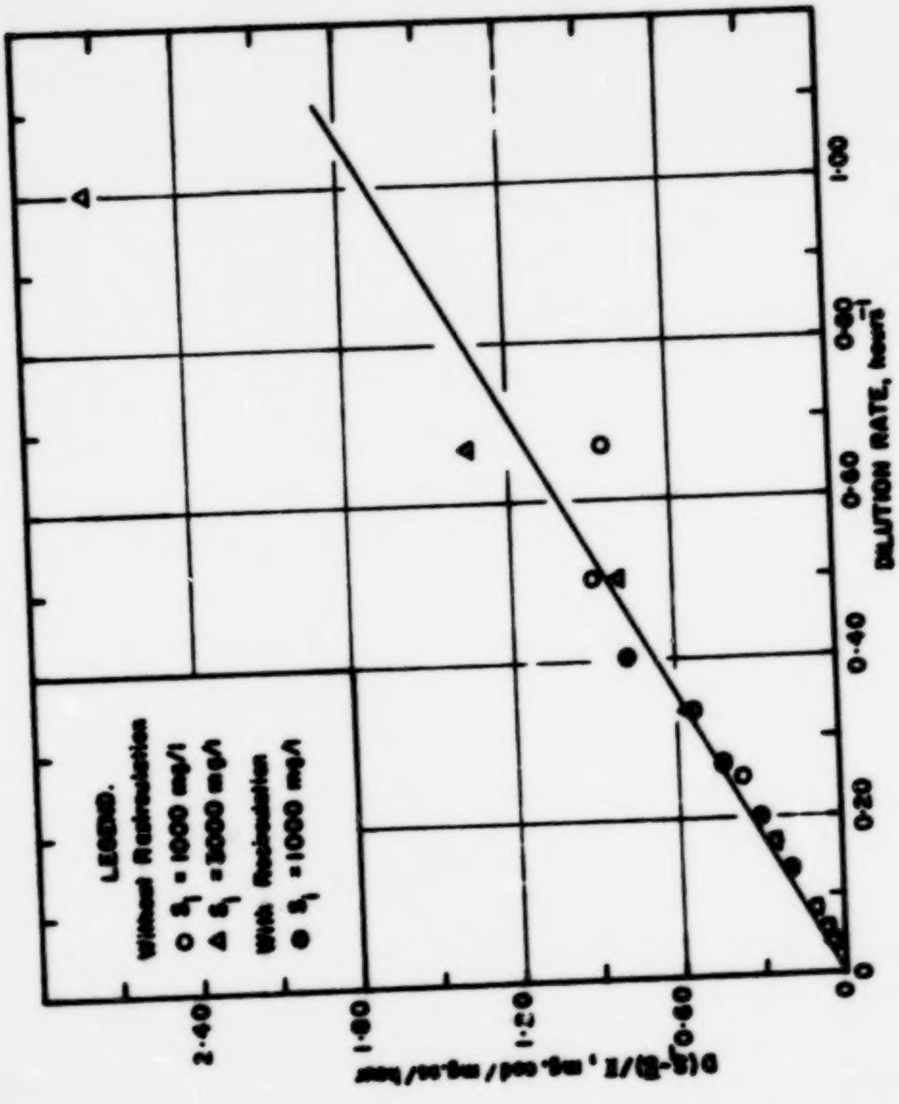
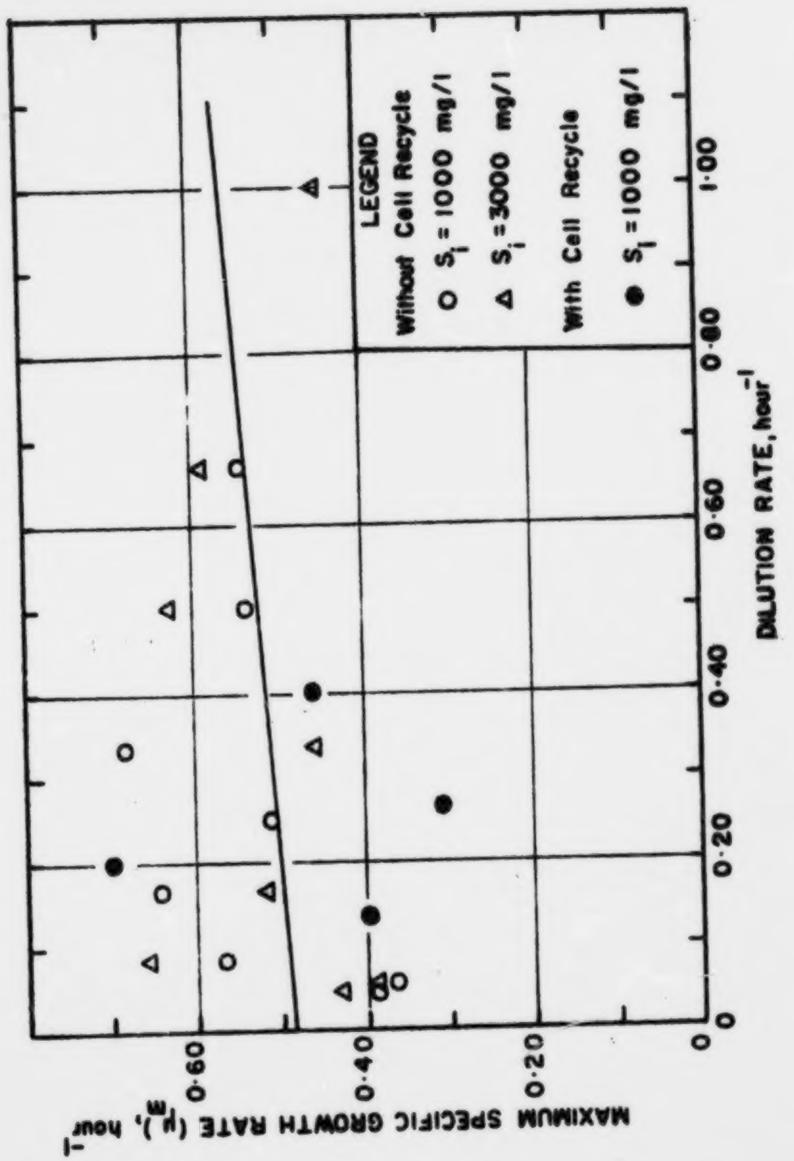

LEGEND.
Without Recirculation
○ S_i = 1000 mg/l
△ S_i = 3000 mg/l
With Recirculation
● S_i = 1000 mg/l
0
0·20
0·40
0·60
0·80
1·00
DILUTION RATE, hour⁻¹
0·60
1·20
1·80
2·40
$D(S_i - S)/X$, mg. cod/mg.ss/hour

Figure 9 - Effect of dilution rate on the value of maximum specific growth rate, μ_m.


LEGEND
Without Cell Recycle
○ S_i = 1000 mg/l
△ S_i = 3000 mg/l
With Cell Recycle
● S_i = 1000 mg/l
MAXIMUM SPECIFIC GROWTH RATE (μ_m), hour^{-1}
DILUTION RATE, hour^{-1}
0
0·20
0·40
0·60
0·80
1·00

Cell output and waste sludge production

Cell output has been defined by Herbert as the product of dilution rate and cell concentration in the effluent (3). For once-through reactor systems, this value, $D\bar{x}$, is given in mg/l/hr. For systems employing cell recycle, cell output was given as the product of D and the cell concentration, x_2, in the final effluent of the separator (in his example, a continuous centrifuge). Since x_2 is equal to $\bar{x}A$, the cell output could be calculated as $D\bar{x}A$ (Equation 14).

In the present work pertaining to the activated sludge process, the sludge was separated by quiescent settling rather than centrifugation, and the aim in this process is to have as few cells in the effluent as possible; in the present experimentation, good sludge settling was attained. The cell output as calculated by Herbert, therefore, has less significant meaning in the case of mixed liquor separation by sedimentation, but if one assumes that the product $D\bar{x}A$ simply represents the mg/l cells/hr which would have gone to the receiving stream if the sludge were not efficiently settled, the cell output is a measure of the amount of excess sludge which was produced. A more useful parameter would be the actual weight of excess sludge produced per hour, i.e., the cell output multiplied by the volume, V, of the aeration chamber. Prediction of the amount of excess sludge produced is as important as the prediction of the concentration of substrate in the effluent or cells in the aerator, since facilities for sludge handling and disposal are integral parts of activated sludge plants. The average cell output (mg/l/hr) and excess sludge produced (mg/hr) for the previous study and the present study are given in Table VI. In general, it is seen that the cell output or waste sludge production increases as D increases, since $\bar{x}$ (see Figure 8 of reference 1, and Figures 5 and 7 of the present report) does not decrease rapidly until dilution rates close to the value of μ_m are approached. As D approaches μ_m, $\bar{x}$ begins to decrease sharply and the cell output passes through a maximum value and falls off sharply. It is noted that the cell output for the system operated at an S_1 of 1000 mg/l without cell recycle, increased at a dilution rate of 1/1.5 hr^{-1}. This inconsistency arises because of the "tailing off" effect.

DISCUSSION

Relation between Growth Rate Constant and Substrate Concentration

In the present study as well as in the study previously reported (1), the cells harvested from the continuous flow reactors at the various dilution rates were used in batch shaker flask experiments to determine the growth rate constant, μ, at various concentrations of substrate. In all cases, the Monod relationship (i.e., $\mu = \mu_m \frac{S}{k_s + S}$ provided a more satisfactory fit of the experimental data than did either the model proposed by Moser (7) or by Teissier (8)(9). It is noted that the Monod equation describes a rectangular hyperbola; accordingly as substrate

TABLE VI

COMPARISON OF CELL OUTPUT AND RATE OF EXCESS SLUDGE PRODUCTION FOR COMPLETELY-MIXED REACTORS WITH AND WITHOUT CELL RECYCLE

Dilution Rate (D) hour^{-1}		S_1 = 3000 mg/l		S_1 = 1000 mg/l			
		Without Cell Recycle		Without Cell Recycle		With Cell Recycle	
		Output mg/l/hr	Excess Sludge mg/hr	Output mg/l/hr	Excess Sludge mg/hr	Output mg/l/hr	Excess Sludge mg/hr
1/24	0.042	67	168	23	58	-	-
1/18	0.056	113	283	38	95	-	-
1/12	0.083	166	415	54	135	-	-
1/6	0.133	-	-	-	-	95	239
1/7.5	0.167	320	800	88	220	-	-
1/5	0.200	-	-	-	-	137	343
1/4	0.250	-	-	165	413	-	-
1/3.75	0.267	-	-	-	-	177	443
1/3	0.333	576	1440	169	423	-	-
1/2.5	0.400	-		-	-	164	410
1/2	0.500	893	2233	51	128	-	-
1/1.5	0.667	451	1128	94	235	-	-
1	1.000	122	305	-	-	-	-

concentration increases, μ does not increase linearly up to μ_m (a discontinuous function) but increases at a decreasing rate as a continuous hyperbolic function. A linear relation was suggested by Garrett and Sawyer (10) as a practical expedient, but has been used by others in developing kinetic theory (11)(12). It has been previously pointed out that when k_s is small, e.g., 10-20 mg/l, it may be possible to approximate the hyperbolic function with a straight line or discontinuous function (see Figure 10 of reference 1).

Comparison of μ_m, k_s, and Y Values

In the previous study (1) using once-through systems fed 1000 mg/l glucose, the average values for μ_m and k_s were 0.53 hr^{-1} and 90 mg/l, respectively, and the average yield value for batch determinations (Y_B) was 0.45 mg/mg substrate; for yields obtained from the continuous flow data (Y_C), the average value was 0.65 mg/mg substrate. In the present study, the corresponding values for the once-through system fed 3000 mg/l glucose were μ_m, 0.52 hr^{-1}, k_s, 57 mg/l, Y_B, 0.47 mg/mg substrate, and Y_C, 0.57 mg/mg substrate. As in the previous study, there was a considerable degree of fluctuation about the average values. In general, it may be concluded that the feed concentration had little or no effect on the values of the "kinetic constants." The average value of the constants for systems employing cell recycle were: μ_m, 0.47 hr^{-1}, k_s, 67 mg/l, Y_B, 0.50 mg/mg substrate, and Y_C, 0.70 mg/mg substrate, and again there was a considerable degree of variation about these values. From the results obtained, it would appear that cell recycle, at the flow rate and concentration factor used in these studies, did not exert a significant effect on the numerical value of the constants. It is interesting to note that in all three sets of experiments the value of cell yield obtained from the continuous flow data is higher than the value obtained from the batch experiments. There is no immediately apparent explanation for this phenomenon, but as stated in the previous report (1), it should be noted that the average values for Y_C at each dilution rate were based upon many more analyses than were the values for Y_B. Therefore, the Y_C values would appear to provide a better assessment of the sludge yield.

In the previous work a study of the data indicated that, in general, for a readily available carbohydrate (e.g., glucose), k_s values in the range of 75-125 mg/l and μ_m values of 0.5-0.6 hr^{-1} can be expected with yield coefficients approximating 0.6 mg/mg substrate. The general range for the present study is consistent with the previous results. It is felt that this range of values can be used with a considerable degree of confidence as a guide for industrial wastes consisting primarily of carbohydrate (e.g., pulp and paper and sugar refinery wastes). Based upon experience gained during the conduct of this research, it is recommended that cell yield values obtained in pilot plant studies usually run to obtain design and operational criteria for industrial waste treatment plants be based upon the "steady state" or continuous flow data. This recommendation is based upon the fact that the yield values obtained from the continuous flow data in the present study were consistently found to be higher than yields based on the batch data. Also, an investigator will be able to collect a large amount of "steady state" yield data since samples for biological solids and substrate removal must be run as a matter of course in operating the pilot plant to determine process efficiency at various loadings, whereas separate batch experiments must be run to obtain Y_B values. Separate batch studies using cells harvested from the continuous flow reactor should be run to obtain average values for μ_m and k_s, but these usually can be made using only optical density readings (appropriately converted to mg/l) to assess growth. The values of μ_m and k_s can be obtained from the steady state data. The value D, at which cell dilute-out occurs, is approximately equal to μ_m, and k_s can be calculated from Equation 12 at various dilution rates. However, this procedure for obtaining μ_m is not recommended because of the "tailing-off" effect which was noted in these experiments. This phenomenon can lead to falsely high values of μ_m. Also, such a procedure is not advisable, because high dilution rates (those near the dilute-out range) tend to select faster growing species which may not be representative of the population present at dilution rates of importance in the final design recommendation. Since one would not wish to design an activated sludge plant which would run at dilution rates so high that the system was on a steep portion of the dilute-out curve (high substrate leakage) it seems more reasonable to determine μ_m and k_s values for populations existing at D values grouped more closely around reasonable corresponding reactor detention times.

The question of selection of reasonable dilution rates (or detention times) for design purposes is indeed an important one. The prime criterion for such selection is without doubt substrate (COD or BOD) removal efficiency. On this basis it was seen in the previous work employing once-through systems (1000 mg/l glucose)(1) that substrate leakage began to increase rather rapidly between D values of 0.25 and 0.30 hr^{-1}. Since μ_m values vary in general between 0.5 and 0.6 hr^{-1}, it was recommended that as a guide, D values of approximately 1/2 of μ_m be used. Regardless of the sharpness of the curves for cell dilute-out and substrate leakage, it is important not to operate too close to the μ_m values, since the only "natural" way in which a completely mixed system can respond to periodic increases in incoming substrate concentration (shock loadings) is by an increase in μ during the ensuing transient state. If D is already close to μ_m, the natural steadying mechanism of completely mixed systems is lost. There are other ways to alleviate substrate leakage during shock load increases in S_i but these, for the most part, require that the operator have knowledge beforehand that the shock is being applied, and such is not usually the case. In the present study it was seen that for the once-through system operated at an S_i of 3000 mg/l glucose, excessive substrate leakage in the effluent did not occur at D values below 0.5 hr^{-1}. Although the removal efficiency was nearly 93 per cent at this dilution rate, the effluent COD was nearly 225 mg/l, approximately 100 per cent higher than at a D value of 0.33 hr^{-1}. Since the value of μ_m was approximately in the same range as for the previous study (1) it is felt that the previous recommendation, while somewhat conservative, should not be revised. Also, this conclusion is altered only slightly by the results of operations employing cell recycle, at least for values of hydraulic recycle ratio (α) and sludge concentration factor (c) employed in the present study. It was seen (Figure 3 and Table III) that substrate leakage was rather low at a D of 1/3.75 hr^{-1} (i.e., a detention time in the aerator of three hours), which is in excess of one half of the average μ_m value. This result is predictable by the reactor theory of Herbert (3) and it is one of the advantageous characteristics of cell recycle systems that operation at D values even in excess of μ_m are possible. In such systems the maximum dilution rate is not only controlled by μ_m but by the value of A, which is determined by the constants α and c (i.e., $A = 1 + \alpha - C\alpha$). In the present study, using $\alpha = 0.25$ and $c = 1.5$ (nominally), the value of A was 0.875 (nominally). It can be seen in Equation 12 that, in accordance with the kinetic theory, if cell recycle is practiced, the system can be operated at higher dilution rates without excess substrate leakage, and, in general, this effect is observable when comparing the dilute-out curves (see Figure 8 of reference 1, and Figure 7, this report). Depending upon the values of α and c employed, cell recycle can have more dramatic effects on the dilute-out curves (see Herbert's hypothetical example calculations, Figure 8, reference 3).

In the present study, a value of 0.25 was chosen for α because it is a recycle ratio often employed for activated sludge processes. The value of c (1.5) was chosen because at the outset of the work it was thought to be a value which could be attained with a minimum of experimental difficulty. It is a lower value than one would expect to employ in an activated sludge process, but the prime purpose of the present study was to test, for heterogeneous microbial populations (or to modify, if need be), the kinetic theory of completely mixed reactors which was derived essentially for the more idealized case (pure cultures). Also, in the interests of making the work more applicable to activated sludge processes, quiescent settling was employed for sludge separation and concentration, and there was no assurance that good settling would always occur. However, a c value of 1.5 might always be readily obtained. As it later developed, the sludge did settle excellently, and a higher value of c could have been used. It is interesting to note that for an α value of 0.25, the value of c can approach but not exceed 5 (i.e., $\alpha = 1 + 0.25 - (0.25)5 = 0$). Under field conditions usually employed, activated sludge may be expected to concentrate four or five times (i.e., settle to 20 or 25 per cent of the original mixed liquor volume) in a reasonable settling time (1-2 hours). This is a fortuitous operational circumstance if sludge is not stored in the secondary clarifier (which is not a good practice in any event), or if the sludge is not otherwise concentrated before recycle. If the sludge is thickened prior to recycle, lower values of α may be employed, thus conserving aeration tank capacity.

Possible modifications to the "steady state" equations for application to the activated sludge process

It was noted in the previous study (1) and in the present study that the biological solids concentration, using heterogeneous populations, is subject to rather large fluctuations. If a constant value of c is to be maintained, the concentration of recycle sludge must therefore be changed daily or, ideally, more often. Operation at a constant concentration of recycle sludge instead of a constant value for c would be more easily facilitated. Indeed, it would seem that such a mode of operation might tend to lessen the usual fluctuations in the concentration of biological solids in the aeration tank. Such an operational procedure was not employed in these studies, because the primary aim was to assess for heterogeneous populations the kinetic formulation of Herbert (3). Nonetheless, operation with a constant concentration of recycle solids seems a better procedure for activated sludge processes. It does, however, require modification of the steady state equations for $\bar{x}$ and $\bar{S}$.

The following expressions for the steady state concentrations of solids, $\bar{x}$, and substrate, $\bar{S}$, when the concentration of recycle solids, x_R, is held constant, can be derived from the mass balance equations for x and S. In deriving the equations given below, it was assumed that the concentration of S in the recycled solids was negligible.

$$\bar{x} = \frac{Y\left[S_i - (1+\alpha)\bar{S}\right] + \alpha x_R}{(1+\alpha)} \qquad (16)$$

The derivation of the equation for the steady state concentration $\bar{S}$ leads to the quadratic form:

$$\bar{S} = \frac{-b \pm \sqrt{b^2 - 4ac}}{2a} \qquad (17)$$

where

$$a = \left[\mu_m - (1+\alpha)D\right] \qquad (18)$$

$$b = D\left[S_i - (1+\alpha)k_s\right] - \frac{\mu_m}{(1+\alpha)}\left[S_i + \frac{\alpha x_R}{Y}\right] \qquad (19)$$

$$c = k_s(D)S_i \qquad (20)$$

Presently we are detemining the kinetic characteristics of these equations.

ACKNOWLEDGEMENT

This work was supported by a research grant WP-00325 from the Federal Water Pollution Control Administration, U. S. Department of the Interior.

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PAPER 18

Goel, K. C., and Gaudy, A. F. Jr., "Regulation of Nitrogen Levels for Heterogeneous Populations of Sewage Origin Grown in Completely Mixed Reactors." (Submitted for publication in Bioengineering and Biotechnology, 1968.)

REGULATION OF NITROGEN LEVELS FOR HETEROGENEOUS POPULATIONS OF SEWAGE ORIGIN GROWN IN COMPLETELY MIXED REACTORS

by

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SUMMARY

Heterogeneous populations of sewage origin were grown continuously at dilution rates from 1/12 hr^{-1} to dilute-out (1/1 hr^{-1}) using glucose (1000 mg/l) as carbon source and three concentrations of NH_3-N as the nitrogen source (COD/N = 70/1, 40/1, and 25/1). The effects of nitrogen level and growth rate (dilution rate) on substrate removal, biological solids production, cellular carbohydrate and protein, and NH_4^+-N in the effluent were examined. It was found that the optimum level of nitrogen supplementation for the "synthetic nitrogen-deficient waste" employed should not be based solely on the desired effluent quality with respect to COD removal but should include due consideration of reactor detention time (or dilution rate) and the allowable (or desirable) level of nitrogen leakage in the effluent.

INTRODUCTION

The pivotal role of the nitrogen source in attainment of microbial growth has been the subject of much research in the fields of microbiology and biological treatment of aqueous wastes. Without the assimilation of a usable source of nitrogen and its subsequent channelling into the synthesis of vital amino acids, proteins, and nucleic acids, additional viable cells cannot be formed. The perpetuation of the biological waste water purification process depends upon renewal of the viable population. The rate of multiplication of cells would not seem to play the most important role insofar as substrate utilization (i.e., wastewater purification) is concerned, since the rate of substrate elimination is more dependent upon the total number of metabolically active cells present than upon the rate at which they increase. Also, it has been shown by many workers that substrate can be removed rapidly even if no nitrogen is present (1)(2)(3)(4)(5); however, this situation cannot be prolonged indefinitely. In an open steady state system without artificial reseeding (cell feedback) a nitrogen source is an absolute necessity, since such a system depends entirely on cell replication.

It has been shown by Komolrit, Goel, and Gaudy that the substrate assimilation process and the replication process can be physically separated (5). It has also been shown how such physical separation of these life processes can be usefully employed in the treatment of a specific nitrogen-deficient waste (6). However, biological treatment of wastes is generally conducted in a growth environment; many wastes are deficient in nitrogen, and to these wastes ammonia is generally added. Much work bearing upon the appropriate amount of nitrogen supplement for a given strength of waste (i.e., the required BOD or COD to N ratio) has been accomplished (7)(8)(9)(10), since this obviously affects the economics of the treatment process. Other wastes contain an excess of nitrogen, and while this situation does not pose an immediate economic concern in treating the waste, the escape of the excess nitrogen adds to problems of stream enrichment or eutrophication. Possible operating conditions for the treatment plant which might lead to alleviation of this problem are presently not known. It was felt that studies in once-through completely mixed reactors employing heterogeneous populations of sewage origin could shed considerable light on both of these aspects of concern in wastewater treatment, and could provide important basic information on heterogeneous populations for comparison with some of the continuous culture studies performed using pure cultures under conditions in which the nitrogen source was employed as the growth-limiting nutrient (11)(12)(13).

MATERIALS AND METHODS

A diagram of the laboratory reactor is shown in Figure 1. The reactor was made from 4-inch diameter glass tubing, and the effluent launder from a section of 6-inch diameter glass tubing.

Air was diffused into the mixed liquor at a rate of 1600 cc/min; at this air flow rate the volume of reactor liquid under air was 2400 ml. Synthetic waste of known composition was pumped into the reactor at predetermined rates which yielded dilution rates of 1/12, 1/4, 1/2, and 1/1 hr^{-1}. The composition of the synthetic wastewater employed in all studies is given in Table I. Glucose at 1000 mg/l was employed as the carbon source, and continuous

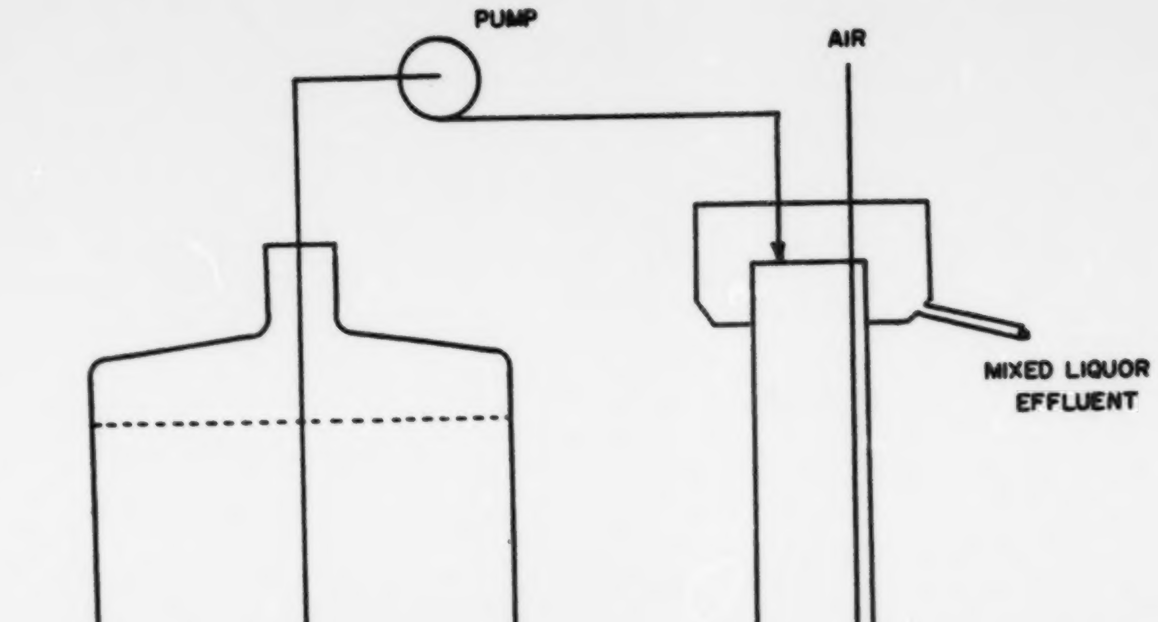

Figure 1 - Schematic drawing of completely mixed continuous flow reactor (chemostat) of the once-through type employed in study on effect of nitrogen levels.

flow operation at three levels of ammonium sulfate, yielding COD/N ratios of 70/1, 40/1, and 25/1 were examined.

TABLE 1

COMPOSITION OF SYNTHETIC WASTE

Constituent	Concentration	COD	COD/N
Glucose	1000 mg/l	1060 mg/l	
$MgSO_4 \cdot 7H_2O$	100 mg/l		
$CaCl_2 \cdot 2H_2O$	7.5 mg/l		
$MnSO_4 \cdot H_2O$	10 mg/l		
$FeCl_3 \cdot 6H_2O$	0.5 mg/l		
KH_2PO_4	527 mg/l		
K_2HPO_4	1070 mg/l		
Tap Water	100 ml/l		
Ammonium Sulfate $(NH_4)_2SO_4$	70 mg/l		70/1
	125 mg/l		40/1
	200 mg/l		25/1
Distilled Water	to 1 liter		

At the initiation of each series of experiments, the reactors were filled with synthetic waste and seeded with a heterogeneous microbial population obtained from the effluent of the primary clarifier at the Stillwater municipal wastewater treatment plant. After the population began to grow, the synthetic waste was pumped in continuously. The system was adjudged to be completely mixed when both the optical density and the substrate concentration in the reactor and the reactor effluent were equal and adjudged to be in the "steady state" when the effluent substrate level remained steady. The unit had previously been shown to be a completely mixed reactor on the basis of coincidence of the theoretical and the observed dilute-in curves at various dilution rates. Under this condition of operation the dilution rate, D, is equal to the growth rate, μ, and to the reciprocal of the reactor detention time, t.

After the system had been operating in the "steady state" for twenty-four hours, samples were collected at periodic intervals for an extended period (usually not less than two weeks) in order to determine operational behavior. Throughout the entire study frequent checks (by optical density) were made to ensure that the cell concentration in the reactor and the effluent were the same. During the studies the average temperature ranged between 22° and $25^{\circ}C$ with a temperature variation at any specific dilution rate of $\pm 1^{\circ}C$. The pH was maintained at 6.8 ± 0.2. Dissolved oxygen in the reactor was determined (electrometrically) periodically during each run and, at the airflow rate and feeding level employed in these studies, was maintained at approximately 5.5 mg/l.

Samples of the effluent mixed liquor were filtered and the concentration of biological solids determined using the membrane filter technique (pore size 0.45μ). The protein (biuret) and carbohydrate (anthrone) content of the biological mass was also determined (14). The membrane filtrate was analyzed for chemical oxygen demand (COD) (15), and for carbohydrate (anthrone) concentration. The presence of various volatile acids in the filtrate was examined by gas chromatography ("polypak" column). The filtrate was also analyzed for ammonia concentration using the method of Niss as described by Ecker and Lockhart (16).

RESULTS AND DISCUSSION

The results presented in Figure 2 show the variation in the "steady state" typical of the experimental runs made during these studies. These data were obtained during a two-week period with the reactor maintained at a dilution rate of 0.083 hr^{-1} and at a COD/N ratio of 70/1. The values for biological solids, effluent COD, etc., for all subsequent analyses and interpretations of system behavior at each dilution rate and COD/N ratio were obtained by calculating average values from data such as those shown in Figure 2. These average data for all experiments are summarized in Table II.

From Figure 2 it is apparent that there were fluctuations in biological solids concentration but, in general, relatively stable values for each parameter were obtained. As would be expected under this rather severe nitrogen limitation, the amount of protein produced was rather low (less than 100 mg/l), but it is interesting to note that the biological solids concentration indicates a rather high

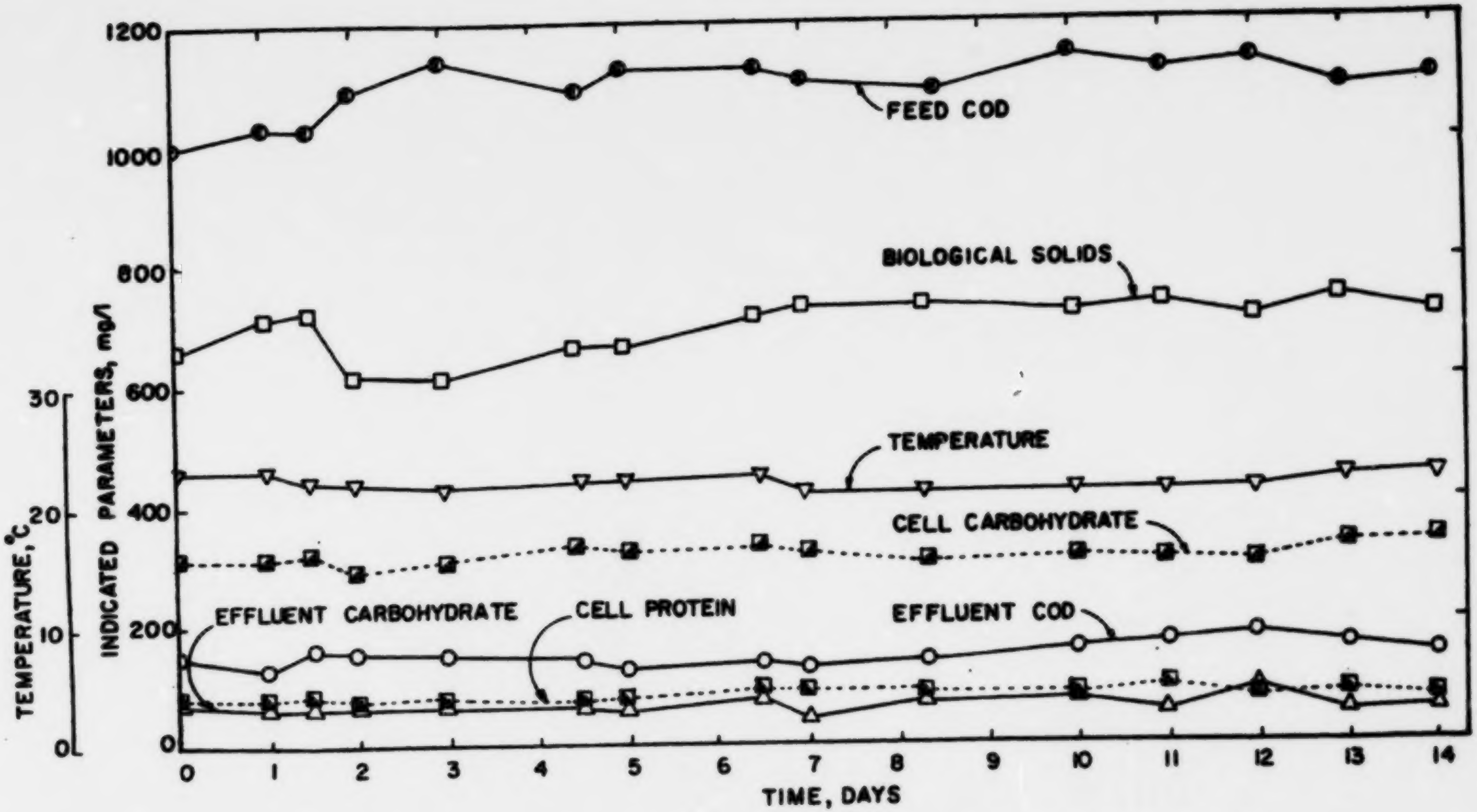

Figure 2 - "Steadv state" parameters in the chemostat during operation at $D = 0.083\ hr^{-1}$ and COD/N = 70/1.

TABLE II

SUBSTRATE REMOVAL, GROWTH AND SLUDGE COMPOSITION FOR HETEROGENEOUS MICROBIAL POPULATIONS GROWN IN A CONTINUOUS FLOW COMPLETELY MIXED REACTOR AT VARIOUS CONCENTRATIONS OF AMMONIA-NITROGEN

Detention Time Hours	Dilution Rate Hour^{-1}	Effluent COD mg/1	COD Removal Efficiency %	Effluent Anthrone COD mg/1	Total Intermediate Products Col 3 - Col 5 mg/1	Products Detected by GLC: Acetic Acid mg/1	Products Detected by GLC: Propionic Acid mg/1	Products Detected by GLC: Ethanol mg/1	Biological Solids mg/1	Yield %	Sludge Carbohydrate mg/1	Sludge Carbohydrate %	Sludge mg/1	Protein %	NH_3-N in Effluent mg/1	NH_3-N Utilized %
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)	(11)	(12)	(13)	(14)	(15)	(16)	(17)
						COD/N in the Feed = 1060/15.1 = 70/1										
1	1.0	996	6	-	-	-	-	-	40	64	-	-	-	-	-	-
2	0.5	823	22	94	729	86	7	50	222	80	110	50	80	36	0.0	100
4	0.25	582	45	90	492	15	0	5	284	55	131	46	80	28	0.0	100
8	0.125	240	77	72	168	15	0	5	690	80	266	39	85	12	0.0	100
12	0.083	150	86	60	90	0	0	0	694	82	314	46	80	12	0.0	100
						COD/N in the Feed = 1060/26.5 = 40/1										
1	1.0	923	13	-	-	-	-	-	58	69	-	-	-	-	-	-
2	0.5	373	65	60	313	17	0	0	336	48	122	36	130	39	2.2	92
4	0.25	180	83	50	130	0	0	0	371	43	128	35	140	38	1.9	93
8	0.125	127	88	32	95	0	0	0	510	55	159	31	125	26	2.2	92
12	0.083	81	92	20	61	0	0	0	709	73	218	31	130	18	2.7	90
						COD/N in the Feed = 1060/42.4 = 25/1										
1	1.0	895	16	-	-	-	-	-	55	53	-	-	-	-	-	-
2	0.5	294	72	42	252	15	0	0	422	60	128	30	215	51	2.7	94
4	0.25	130	88	15	115	0	0	0	400	46	113	28	200	50	3.8	91
8	0.125	63	94	15	48	0	0	0	394	42	97	25	180	48	6.2	86
12	0.083	57	95	10	47	0	0	0	384	62	147	25	175	30	8.6	80

"cell" yield; a considerable portion of the biological solids consisted of carbohydrate. It is also interesting to note that, at this reactor detention time (twelve hours) the COD removal efficiency was rather good.

Figure 3 shows average biological solids concentration and effluent COD concentrations for all studies carried out at a COD/N ratio of 70/1. At this COD/N ratio, the system was subject to rather severe "dilute out"; under operation at D = 0.25 (4-hour detention time) the substrate removal efficiency was very poor.

Examination of Table II reveals some interesting and significant facts worthy of analysis from both the basic and applied viewpoints concerning the behavior of heterogeneous (or natural) populations. It was felt that interpretative analyses of these data could be enhanced by separate consideration as well as correlative consideration of the parameters. Accordingly, values for the various parameters shown in Table II are plotted vs. the concentration of ammonia nitrogen in the feed (or COD/N ratio) at each dilution rate in Figures 4-9. The gas liquid chromatographic analyses do not lend themselves to graphical presentation. Although the column employed was capable of retaining many compounds, acetic acid, propionic acid, and ethanol were the only ones detected, and of these, only acetic acid was found at all COD/N ratios employed. Significant amounts of acetic acid and ethanol were detected at the 70/1 level when the system was operated at the 0.5 hr^{-1} dilution rate.

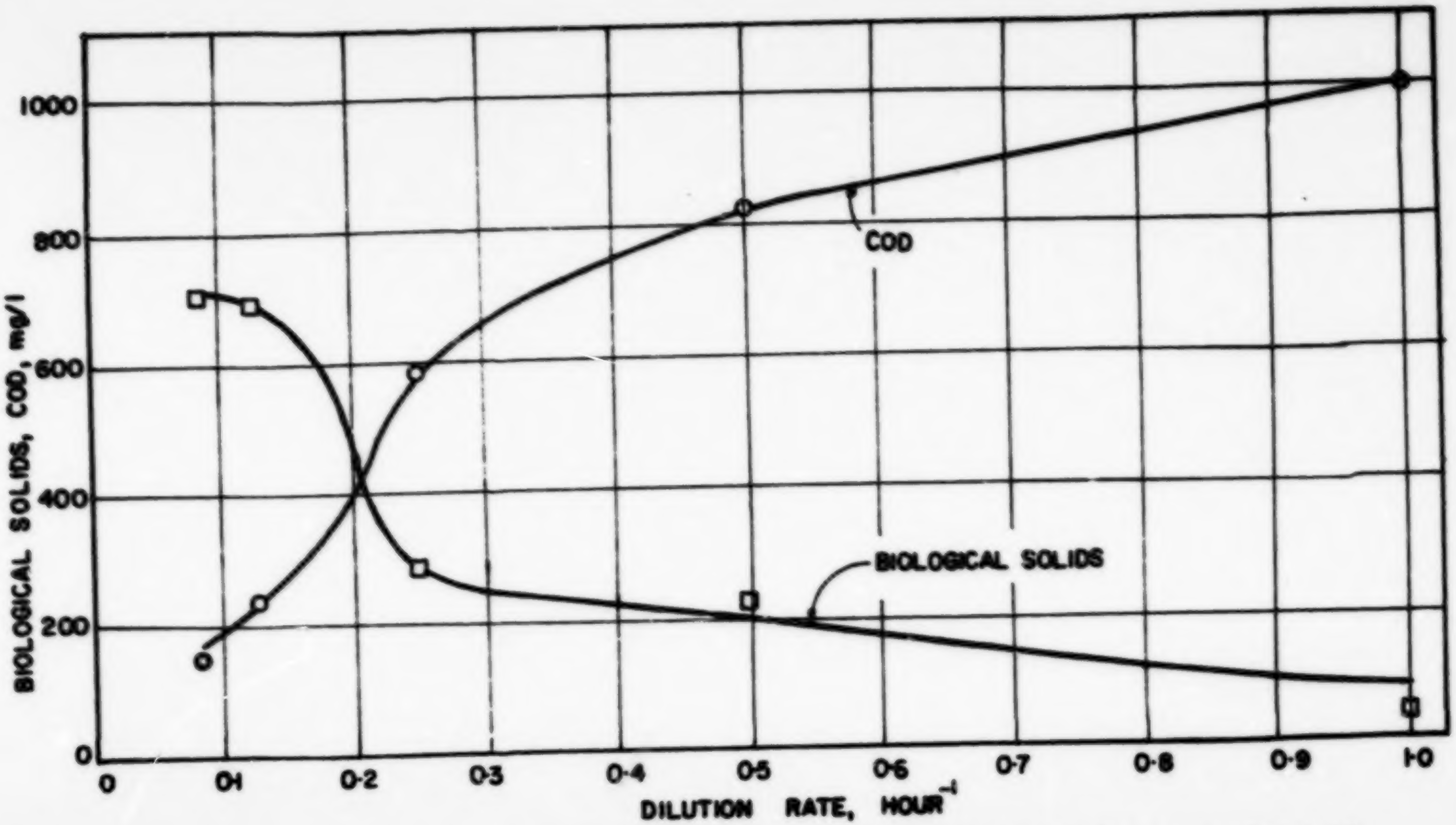

Figure 3 - Dilute-out curves of biological solids and substrate for operation at COD/N = 70/1.

Although we have often observed the presence of acetic acid in the medium for rapidly growing systems, the presence of ethanol would appear to indicate the possibility of concurrent fermentation and aerobic metabolism under aerobic growth conditions at low nitrogen source levels.

In biological wastewater purification processes, the foremost consideration is, quite naturally, the degree of substrate utilization. The relationship between the nitrogen level in the feed and the effluent COD (as determined by the chemical oxygen demand test) is shown in Figure 4. It is seen that at a dilution rate of 1/1 hr^{-1} the systems were, for all practical purposes, diluted out at any of the nitrogen levels employed, and it is obvious that a self-sustaining (no cell recycle) system cannot be operated efficiently at this detention time (with the minimal medium and the temperature employed in the present experimentation). Previous studies have shown that even a COD/N ratio of 10/1 does not permit efficient operation at this high dilution rate (17).

The effect of dilution rate (or detention time) on effluent quality is greatly magnified at the low nitrogen level (COD/N = 70/1) and diminishes rapidly as the nitrogen level increases, especially at the lower detention times. Increasing the nitrogen level beyond that needed to yield a COD/N ratio of 40/1 would appear to provide a sharply diminished return with respect to effluent quality.

The effect of the nitrogen level on biological solids production is shown in Figure 5. It is seen that the maximum

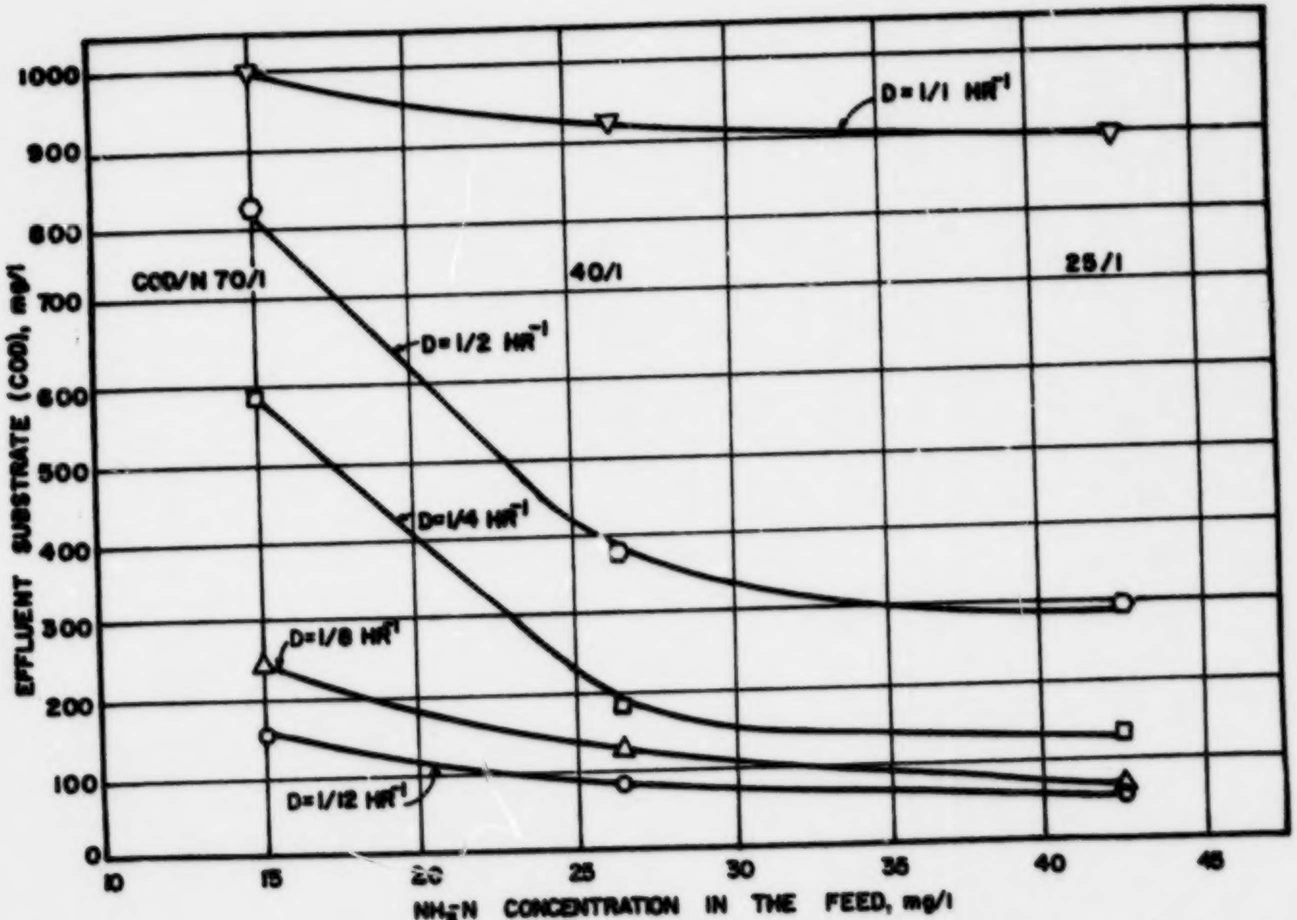

Figure 4 - Relation between effluent COD and NH_3-N (or COD/N ratio) at various dilution rates.

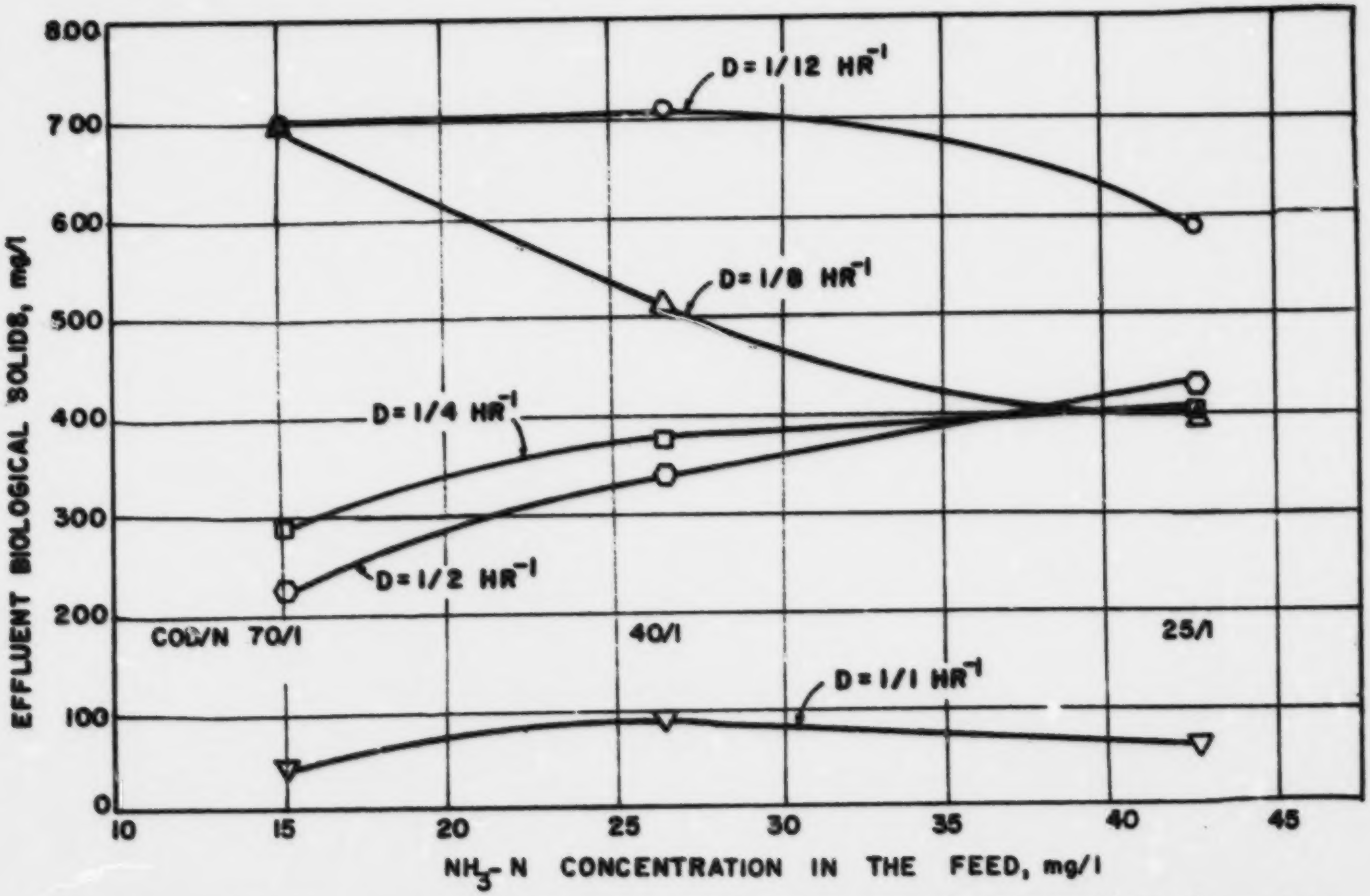

Figure 5 - Effect of nitrogen level on biological solids production at various dilution rates.

biological solids production which was attained occurred at the lowest nitrogen level employed (COD/N = 70/1) for the higher detention times (D = 1/12 and 1/8 hr^{-1}). Also, the minimum solids concentrations which were observed (exclusive of the system which was diluted out) were attained at the COD/N ratio of 70/1 for the low detention times (D = 1/4 and 1/2 hr^{-1}). For the COD/N ratio of 40/1, the sludge concentration at the high detention time (12 hours) was the same as at 70/1, but the presence of the higher level of nitrogen caused a drop in the biological solids level when the cells were made to grow faster (i.e., at D = 1/8 hr^{-1}). On the other hand, as the growth rate was increased further, (D = 1/4 and 1/2 hr^{-1}) the solids levels were higher at lower COD/N ratios. As the system approached more closely to conditions under which carbon rather than nitrogen source was the growth-limiting factor (COD/N = 25/1), the biological solids levels tended to converge, although the biological solids level at the slowest growth rate (D = 1/12 hr^{-1}) remained somewhat higher than for the other systems.

Some insight into the reason for the drastic effect of growth rate (dilution rate) on biological solids production at the low nitrogen level (COD/N = 70/1) is provided by analysis of Figure 6. It is seen that over 300 mg/l of carbohydrate were produced at the higher detention time (lower growth rate), whereas only slightly over 100 mg/l were produced at the lowest detention time. As the COD/N ratio was decreased, there was a drastic reduction in

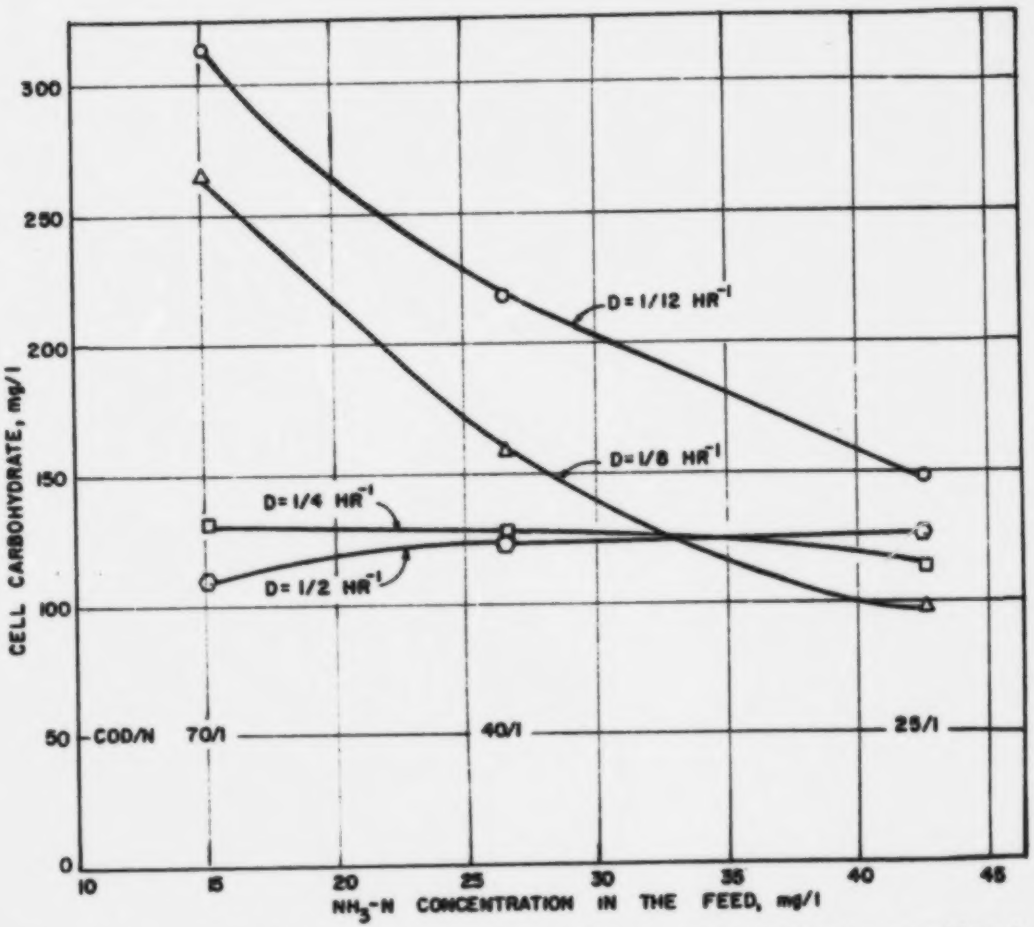

Figure 6 - Effect of nitrogen level on carbohydrate synthesis at various dilution rates.

carbohydrate synthesis at the 12 and 8-hour detention times. As the system approached a COD/N ratio at which carbon rather than nitrogen was the growth-limiting factor (COD/N = 25/1), detention time exhibited a diminished effect on carbohydrate synthesis. It is important to note that at the lower nitrogen levels and higher detention times (corresponding to high cell carbohydrate levels) a large amount of capsular material was produced and the mixed liquor was very viscous. It cannot be said whether these conditions of operation caused an en masse production of extracellular polysaccharide or provided a selective pressure for species which naturally produce massive amounts of capsular material. In any event, as the detention time was decreased from 12 to 8 to 4 hours, at both the 70/1 and 40/1 COD/N ratios, the amount of capsular material decreased; the effect was much more noticeable at the 70/1 COD/N ratio. It is also interesting to note that although there was a decided reduction in the total amount of cellular carbohydrate production as growth rate was increased (i.e., as detention time was decreased) at these COD/N ratios, there was a slight increase in carbohydrate content (percent) of the bio-mass (see Table II).

The pattern of protein production offers further insight into the behavior of these systems (see Figure 7). Detention time had no effect on cellular protein concentration at the low nitrogen level, and the effect at the 40/1 level may for all practical purposes be considered

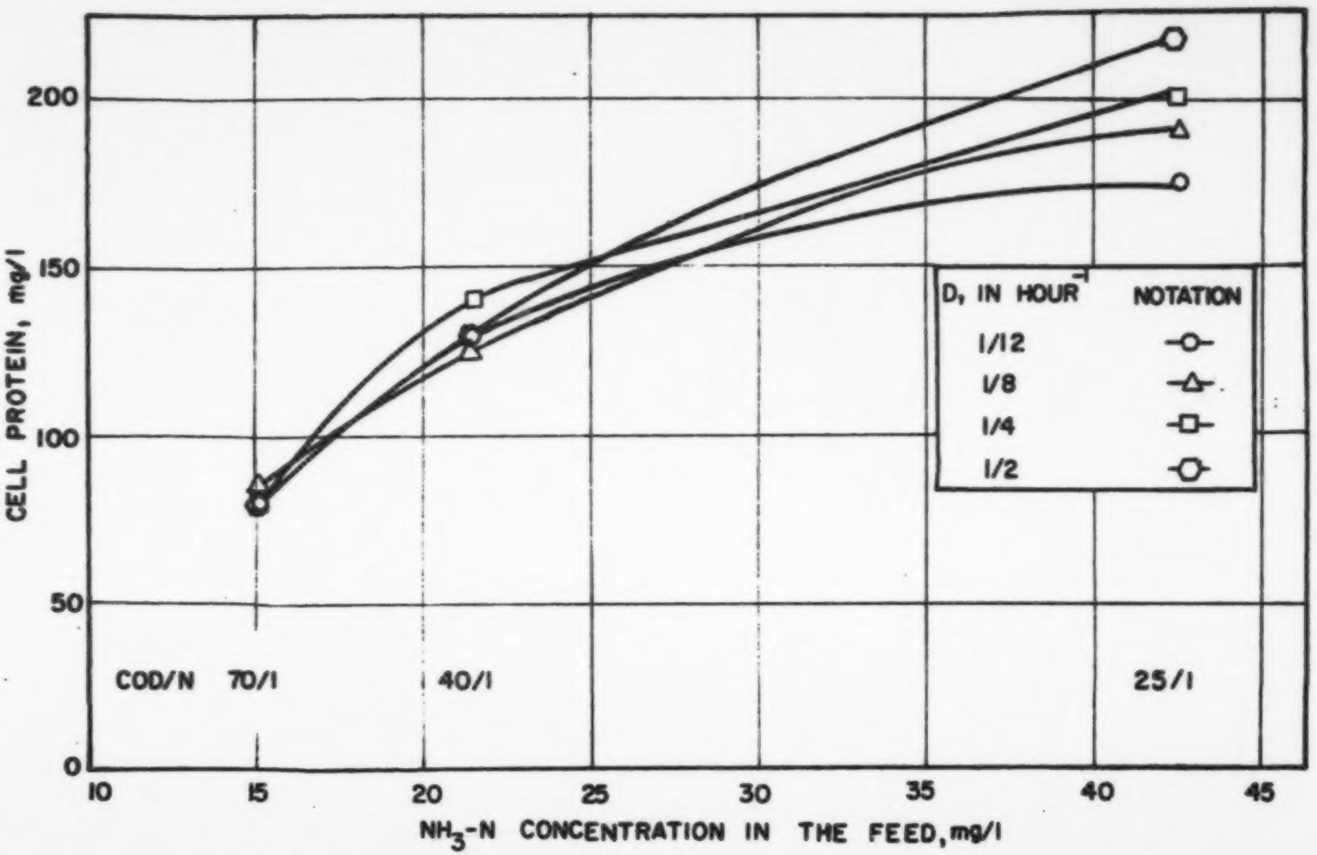

Figure 7 - Effect of nitrogen level on protein synthesis at various dilution rates.

negligible. However, at the highest nitrogen level, the difference in protein synthesis while still rather small, does seem significant, and the trend is definite, i.e., at the higher dilution rates (lower detention times) protein production increased. Such a result seems consistent with behavior one might expect in a system for which the carbon source rather than the nitrogen source was the growth-limiting factor. It would appear from Figures 6 and 7 that while more protein synthesis may be expected at faster growth rates in systems containing higher nitrogen levels, the substrate removal capability of systems operating under rather severe nitrogen limitation can be enhanced by decreasing the growth rate, i.e., increasing the detention time, which allows time for the synthesis of nonessential cellular material (e.g., carbohydrate). In short, it would appear that under conditions of nitrogen deficiency the force feeding of carbon source, in the present case carbohydrate, fosters not only saturation of internal carbohydrate stores (5)(12) but production of extracellular carbohydrate as well. Wilkinson, in pure culture studies, also noted the buildup of both intracellular and extracellular carbohydrate under nitrogen-deficient conditions (18).

The dilution rate also affects the amount of nitrogen leakage in the effluent, as shown in Figure 8. At a COD/N ratio of 70/1, all of the added nitrogen was assimilated (also see Table II). When the COD/N ratio was increased to 40/1, the slowest-growing system ($D = 1/12\ hr^{-1}$) exhibited

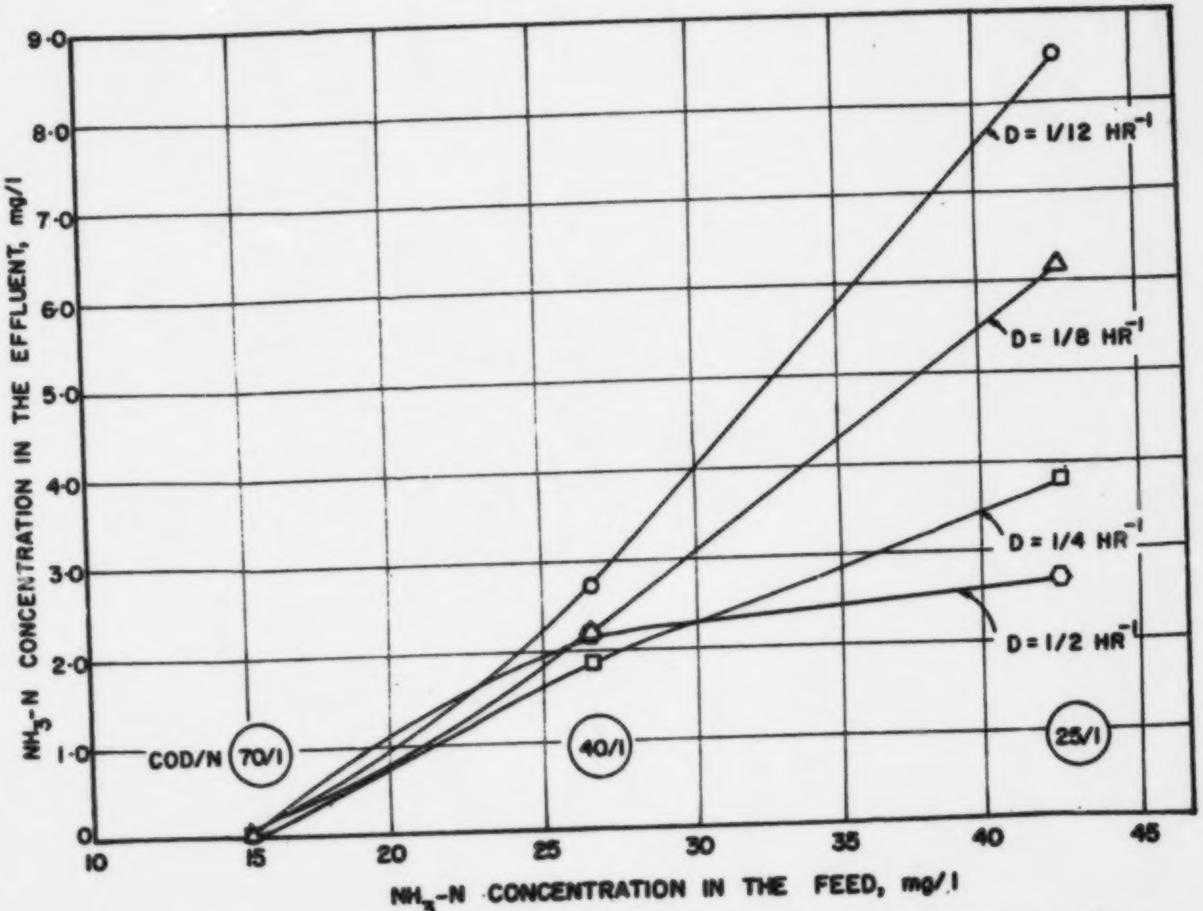

Figure 8 - Effect of nitrogen level on leakage of nitrogen in the effluent at various dilution rates.

the highest concentration of nitrogen in the effluent. When the nitrogen limitation was essentially relieved (COD/N = 25/1) there was a marked spread in the concentration of NH_3-N in the effluent (from 2.7 mg/l at D = 1/2 hr^{-1} to 8.6 mg/l at D = 1/12 hr^{-1}). As was shown in Figure 7, significantly higher concentrations of protein were produced at the faster growth rates. It is interesting to note that, at this nitrogen level (COD/N = 40/1) in the feed, while operation at higher detention times (lower growth rates) enhances removal of carbon source (Figure 4) it also enhances nitrogen leakage in the effluent. In systems which are not limited by the concentration of the nitrogen source, it seems reasonable to expect that at the higher detention times the accompanying lower rate of cell replication leads to synthesis of lower amounts of protein. The lower the amount of protein needed to accommodate steady state growth, the lower would be the utilization of nitrogen source, hence the greater the nitrogen leakage.

From time to time in the pollution control field attention has been drawn to the importance of the nitrogen content of the sludge as a parameter for determining its purification capability (8)(9). In the present study the protein content, rather than the organic nitrogen, was determined. For the purpose of comparing effluent quality at various nitrogen contents of the sludge, the protein content was divided by 6.25 (16.0 per cent N in protein). Figure 9 shows such a comparison using the data obtained in the present study at

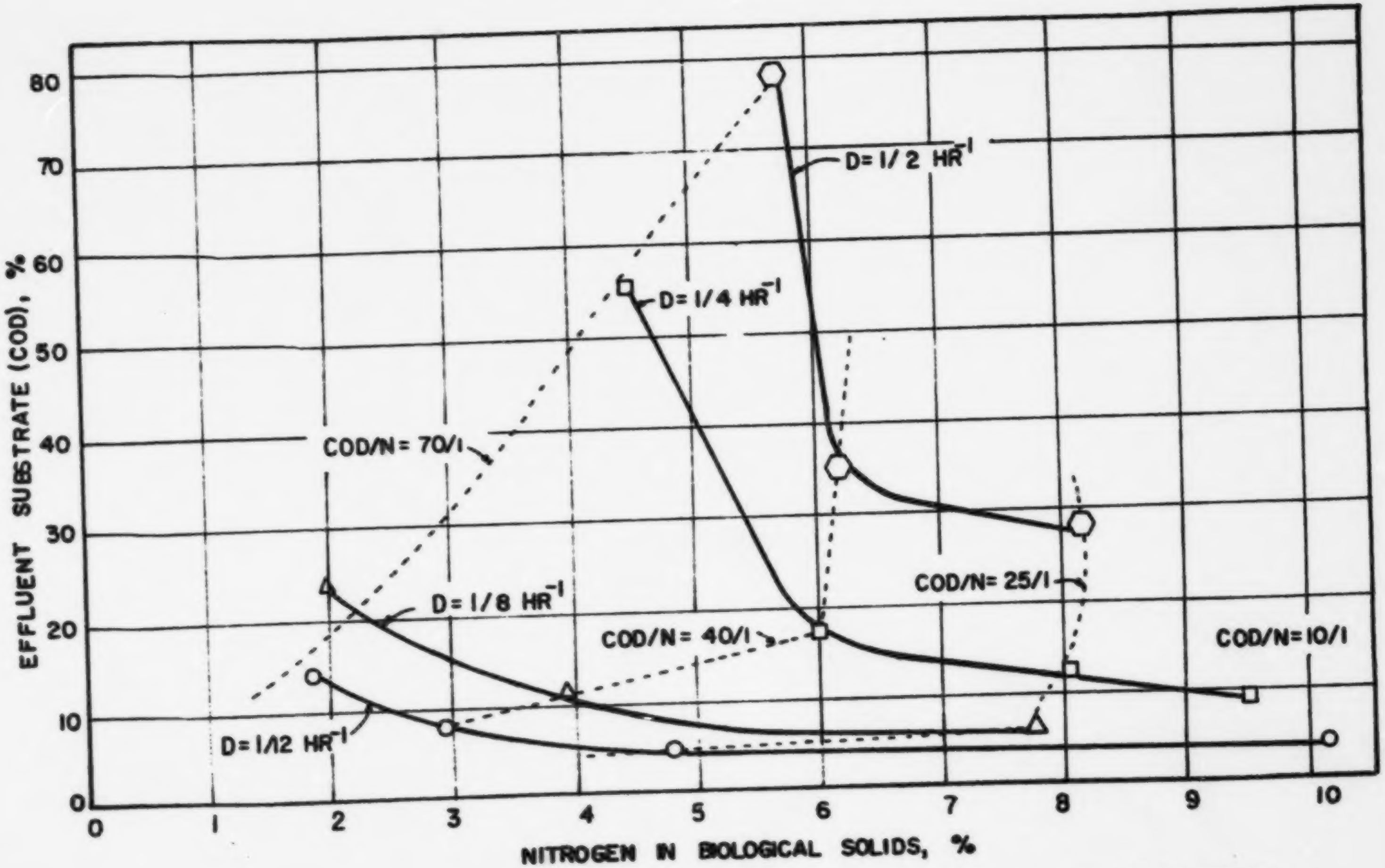

Figure 9 - Relation between effluent COD and nitrogen content of the biological solids at various dilution rates and COD/N ratios.

COD/N ratios of 70/1, 40/1, and 25/1; the data points for the COD/N ratio of 10/1 were taken from a previous study (17). It is seen that under rather severe nitrogen limitation (COD/N = 70/1) the nitrogen content of the sludge can vary from slightly less than two per cent (at D = 1/12 hr^{-1}) to nearly six per cent (D = 1/2 hr^{-1}) depending upon the growth rate. At low nitrogen levels it is re-emphasized that excellent removal of carbon source can be attained at high detention times, whereas detention time becomes a less important factor as the COD/N ratio is decreased. If, for example, one establishes ninety per cent COD removal as the prime criterion for process efficiency, this degree of purification can be accomplished with a 12-hour detention period and a sludge nitrogen content of approximately 2.5 per cent at a COD/N ratio of between 70/1 and 40/1. By decreasing the COD/N ratio to 40/1, the same degree of purification can be attained using an 8-hour detention period and the nitrogen content of the sludge will be slightly in excess of four per cent. The same system efficiency can be attained at a 4-hour detention period and nitrogen content of the sludge of 9 per cent at a COD/N ratio between 25/1 and 10/1. However, the balancing of detention time and nitrogen supplementation is not the only consideration which should be examined. With the present concern over stream enrichment, it is also desirable to consider nitrogen leakage in the effluent. While it is apparent from Figure 9 that higher levels of nitrogen and longer detention times enhance COD removal, this combination of conditions was also seen (Figure 8) to enhance nitrogen leakage in the effluent. From the results shown in Figure 9 it would appear that from the standpoint of COD removal, the nitrogen content of the sludge is not a uniquely applicable parameter and must be considered in the light of the desired (or most economical) detention time. However, it should be emphasized that there are industrial process situations wherein the protein content of the cells may be as important as optimum substrate removal. Also, there are cases in the treatment of certain high strength industrial wastes wherein both considerations are of equal importance. With the advent of production of synthetic foods from nonagriculturally produced proteins, this consideration may assume increasing importance and indeed may provide a means of ameliorating some of the cost of secondary treatment for certain high strength industrial wastes. In any event, it seems apparent from the results of this study that one cannot recommend any one range of nitrogen content as being an ideal one without due consideration of the relationships shown in Figures 8 and 9.

SUMMARY AND CONCLUSIONS

In summary, it has been shown in these studies that the system dilute-out pattern for heterogeneous microbial populations in completely mixed reactors operated under conditions wherein the nitrogen source is the growth-limiting factor, is similar to that observed in pure culture studies (11) (12). Although sludge recycle was not practiced in the present studies, and although substrate or COD removal efficiency was based on filtrate values rather than figures obtained from settled supernatant (i.e., biochemical efficiency vs. total system efficiency, including settling), the results nonetheless provide useful insight into the behavior of, and the nitrogen requirements for, activated sludge as well as any other system employing fluidized heterogeneous microbial populations.

From the results obtained in this study it would seem apparent that the optimum COD/N ratio to be employed for the treatment of organic wastes cannot be selected solely on the basis of the desired effluent quality with respect to carbon removal, since the optimum nitrogen supplementation will be somewhat dependent upon the reactor detention time employed. Also, the amount of nitrogen leakage in the effluent is dependent upon both the detention time and the COD/N ratio. From the present results it appears that nitrogen supplementation in excess of that needed to yield a COD/N ratio of 40/1 does not seem advisable, since nitrogen addition in excess of this value does not add greatly to the efficiency of substrate removal but can contribute significantly to nitrogen leakage in the effluent (compare Figures 4 and 8). For the synthetic waste employed in these studies, the BOD/COD ratio was found (experimentally) to be 0.667. Thus, on the basis of the present results it is recommended that the BOD/N ratio need not be lower than 27/1. This value is slightly higher than that (20/1) usually employed, but is well within the range originally recommended by Sawyer and his co-workers (7)(8)(9). However, it should again be explained that higher concentrations of nitrogen source will provide slightly better carbon removal efficiency (and greater leakage of nitrogen in the effluent), and also that excellent substrate removal can be attained with the use of lower nitrogen concentrations (and greater reactor detention times).

ACKNOWLEDGEMENT

This work was supported by a Research Grant WP 00325 from the Water Pollution Control Administration, U. S. Department of the Interior.

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PAPER 19

Goel, K. C., and Gaudy, A. F. Jr., "Studies on the Relationship between Specific Growth Rate and Concentration of Nitrogen Source for Heterogeneous Microbial Populations of Sewage Origin." (Submitted for publication in Biotechnology and Bioengineering, 1968.)

STUDIES ON THE RELATIONSHIP BETWEEN SPECIFIC GROWTH RATE AND CONCENTRATION OF NITROGEN SOURCE FOR HETEROGENEOUS MICROBIAL POPULATIONS OF SEWAGE ORIGIN

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SUMMARY

Batch experiments were run using heterogeneous populations to determine whether a hyperbolic equation of the type suggested by Monod could be used to depict the relation between specific growth rate, μ, and NH_3-N concentration when ammonia-N was the growth-limiting nutrient. The heterogeneous populations employed were developed from sewage seed grown on glucose at various levels of nitrogen and various dilution rates in completely mixed continuous flow reactors. It was found that the hyperbolic function could be used. Values of μ_m in the range of 0.4-0.7 hr^{-1} were observed, and values of K_s, in general, ranged from 1.5 to 4.0 mg/l. Variation in the values of these growth "constants" did not follow any discernible pattern related to past growth history (i.e., COD/N ratio or dilution rate at which the cells were previously grown).

INTRODUCTION

In a previous study (1) the behavior of heterogeneous populations grown in completely mixed continuous flow reactors (chemostats) of the once-through type were examined at various dilution rates and levels of ammonia-N concentrations in the feed. Substrate concentration in the feed was held constant. It was found that the optimum ratio of chemical oxygen demand of the incoming feed to available nitrogen source, i.e., the COD/N ratio which provided good substrate removal efficiency, was dependent upon the reactor detention time (or dilution rate). Also, the amount of nitrogen leakage in the reactor effluent was found to be dependent upon the COD/N ratio and the reactor detention time. During these studies, advantage was taken of the opportunity to make a separate investigation of the relationship between specific growth rate, μ, and concentration of growth-limiting nutrient when nitrogen source was made the growth-limiting nutrient, using cells taken from the chemostats. These studies are the subject of the present report.

It had been shown previously (2)(3) that for carbon-limited systems, the hyperbolic function suggested by Monod, on the basis of studies with pure cultures, also provided the best description of the relationship between μ and substrate concentration, S, for heterogeneous populations:

$$\mu = \mu_m \frac{S}{K_s + S} \qquad (1)$$

Values of maximum specific growth rate, μ_m, and saturation constant, K_s, are usually considered to be kinetic constants for particular microbial species, substrates and operational conditions (temperature, pH, etc.). They were not constants for heterogeneous populations, but did fall within a range sufficiently narrow to permit useful engineering application. The same general equation may be applied to express the relationship between μ and concentration of the nitrogen source when it is made the growth-limiting nutrient. Since for organotrophic organisms considerably less nitrogen (or other possible growth-limiting nutrient) than carbon source is required for balanced growth, the values for K_s have been found to be very small; values in the range 10^{-6}M have been observed (4). Since steady state equations incorporating the Monod relationship are being increasingly considered for depicting the kinetics of completely mixed aerobic biological treatment processes, it seemed desirable to determine whether the hyperbolic function could be applied to heterogeneous populations when nitrogen was made the growth-limiting nutrient and to determine, if possible, an estimate of the range of K_s values which might be expected. It was also reasoned that such studies could provide a check on the expected range of μ_m values previously reported for heterogeneous populations growing on an easily metabolized carbohydrate (glucose) as carbon source. In both chemostat studies with heterogeneous populations of sewage origin using nitrogen source as growth-limiting nutrient (1) and those using carbon source as growth-limiting nutrient (2)(3), the nitrogen source was NH_3-N and the carbon source was glucose, and operational conditions with respect to temperature, pH, aeration rate, and mineral content of the medium were similar. Therefore μ_m values could be compared.

MATERIALS AND METHODS

Equal portions of synthetic medium of the same composition as used in the chemostats (1) but devoid of carbon and nitrogen source were placed in 250 ml Ehrlenmeyer flasks. Glucose was added to yield a final concentration of 500 mg/l in each flask. Ammonium sulfate was added to yield final concentrations of nitrogen ranging from 2 μg/l to 100 mg/l. All flasks were inoculated with small equal portions of a dilute washed suspension of cells harvested from the chemostat effluent. The flasks were then placed on a reciprocal shaker (100 oscillations per minute) at 25°C. Experiments were run using cells taken from the chemostat during operation at each of three COD/N ratios and all dilution rates employed.

During incubation on the shaker, samples were withdrawn for measurement of optical density. Optical density values could be converted to biological solids concentration using a previously prepared calibration curve. The biological solids concentration was also determined by the membrane filter technique (pore size, 0.45 microns) at the end of the shaker experiments and checked against the calibration curve. The chemical oxygen demand (COD) and the NH_3-N concentration of the filtrate were determined as described previously (1).

RESULTS AND DISCUSSION

During operation at each dilution rate for each of the COD/N ratios, cells were harvested from the completely mixed continuous flow reactors and used in the batch studies described earlier. For operation at the COD/N level of 70/1, the cells were heavily encapsulated (apparently with a polysaccharide) (1). As a result, the optical density data during the batch growth studies could not be confidently employed in drawing conclusions. For this reason, only the results obtained using cells harvested from units operating at COD/N ratios of 40/1 and 25/1 are shown in summarized results given in Table I.

TABLE I

SPECIFIC GROWTH RATE, μ, AND UTILIZATION OF NH_3-N AND SUBSTRATE, FOR HETEROGENEOUS POPULATIONS GROWN IN 500 mg/1 GLUCOSE AT VARIOUS CONCENTRATIONS OF NH_3-N

Source of Cells	Initial NH_3-N	D = 1/2 $hour^{-1}$			D = 1/4 $hour^{-1}$			D = 1/8 $hour^{-1}$			D = 1/12 $hour^{-1}$		
		Final NH_3-N	COD Utilized	μ	Final NH_3-N	COD Utilized	μ	Final NH_3-N	COD Utilized	μ	Final NH_3-N	COD Utilized	μ
		mg/1	%	$hour^{-1}$	mg/1	%	$hour^{-1}$	mg/1	%	$hour^{-1}$	mg/1	%	$hour^{-1}$
Chemostat Operating at COD/N = 40/1	2.0 µg/1	0	9	-	trace	3	-	trace	10	-	trace	5	-
	10.0 "	0	16	-	"	4	-	"	2	-	"	2	-
	20.0 "	0	20	-	"	2	-	"	10	-	"	3	-
S_1 = 1000 mg/1 Glucose	50.0 "	0	40	-	"	4	-	"	5	-	"	2	-
	0.1 mg/1	0	41	-	"	4	-	"	5	-	"	5	-
	0.2 "	0	44	-	-	-	-	"	8	-	"	3	-
Original Seed, Sewage	1.0 "	0	46	0.16	"	11	0.23	"	4	0.04	"	10	0.12
	2.0 "	0	48	0.24	"	21	0.22	"	35	0.10	"	16	0.39
	10.0 "	0	72	0.45	"	62	0.41	"	79	0.67	"	53	0.35
	20.0 "	trace	93	0.45	"	81	0.41	"	87	0.59	"	74	0.39
	50.0 "	12	94	0.45	20	87	0.36	35	80	0.67	36	77	0.43
	100.0 "	>50	93	0.45	>50	83	0.39	>50	93	0.67	>50	72	0.39
Chemostat Operating at COD/N = 25:1	2.0 µg/1	trace	4	-	trace	-	-	trace	5	-	trace	1	
	10.0 "	"	5	-	"	2	-	"	7	-	"	3	
	20.0 "	"	2	-	"	2	-	"	-	-	"	1	
S_1 = 1000 mg/1 Glucose	50.0 "	"	5	-	"	7	-	"	7	-	"	7	
	0.1 mg/1	"	5	-	"	11	-	-	-	-	"	7	
	0.2 "	"	4	-	"	9	-	"	12	-	"	6	
Original Seed, Sewage	1.0 "	"	8	0.14	"	15	0.20	"	11	0.17	"	17	0.15
	2.0 "	"	16	0.29	"	13	-	"	24	0.49	"	21	
	10.0 "	"	62	0.40	"	68	0.37	"	46	0.59	"	77	0.52
	20.0 "	"	73	0.42	"	88	0.37	"	73	0.59	5	86	0.52
	30.0 "	5	-	0.48	-		-	-	-	-	11	87	0.64
	40.0 "	15	76	0.37	-		-	-	-	-	18	89	0.58
	50.0 "	26	72	0.40	35	87	0.29	35	81	0.70	34	87	0.58
	100.0 "	~75	73	0.44	>50	84	0.28	>80	77	0.70	~75	81	0.58

The values of the growth rate constant, μ (shown in Table I), were calculated from optical density (and/or biological solids) data such as shown in Figure 1. The example shown in Figure 1 is a plot of the growth data obtained using cells harvested from the continuous flow reactor operating at a COD/N ratio of 40/1 and a dilution rate of 1/2 hour^{-1}. Similar plots were made for all other systems shown in Table I. In the interest of brevity, only the typical example of Figure 1 is shown. In general, sufficient plotting points to provide a confident estimate of μ were obtained. At ammonia-nitrogen concentrations below 1 mg/1 the data were not sufficient to obtain μ from a plot of optical density values, i.e., growth was so small that it could not be measured by the methods employed. From Figure 1 it is seen that maximum total growth was attained at 20 mg/1 NH_3-N (or above). At this level and above, approximately 275-300 mg/1 biological solids were produced and nearly all of the substrate (see Table I) was consumed. At NH_3-N levels of 10, 2, and 1 mg/1, biological solids production was increasingly curtailed. However, it is interesting to note that the maximum attainable growth rate constant, μ, for this cell population was attained at (or near) the 10 mg/1 NH_3-N level. Thus, the level of growth-limiting material (in the present case, nitrogen source) needed to produce maximum attainable biological solids (or cell) concentration for a given amount of carbon source does not coincide with the concentration of limiting nutrient needed to attain the maximum value of μ. From Table I it is

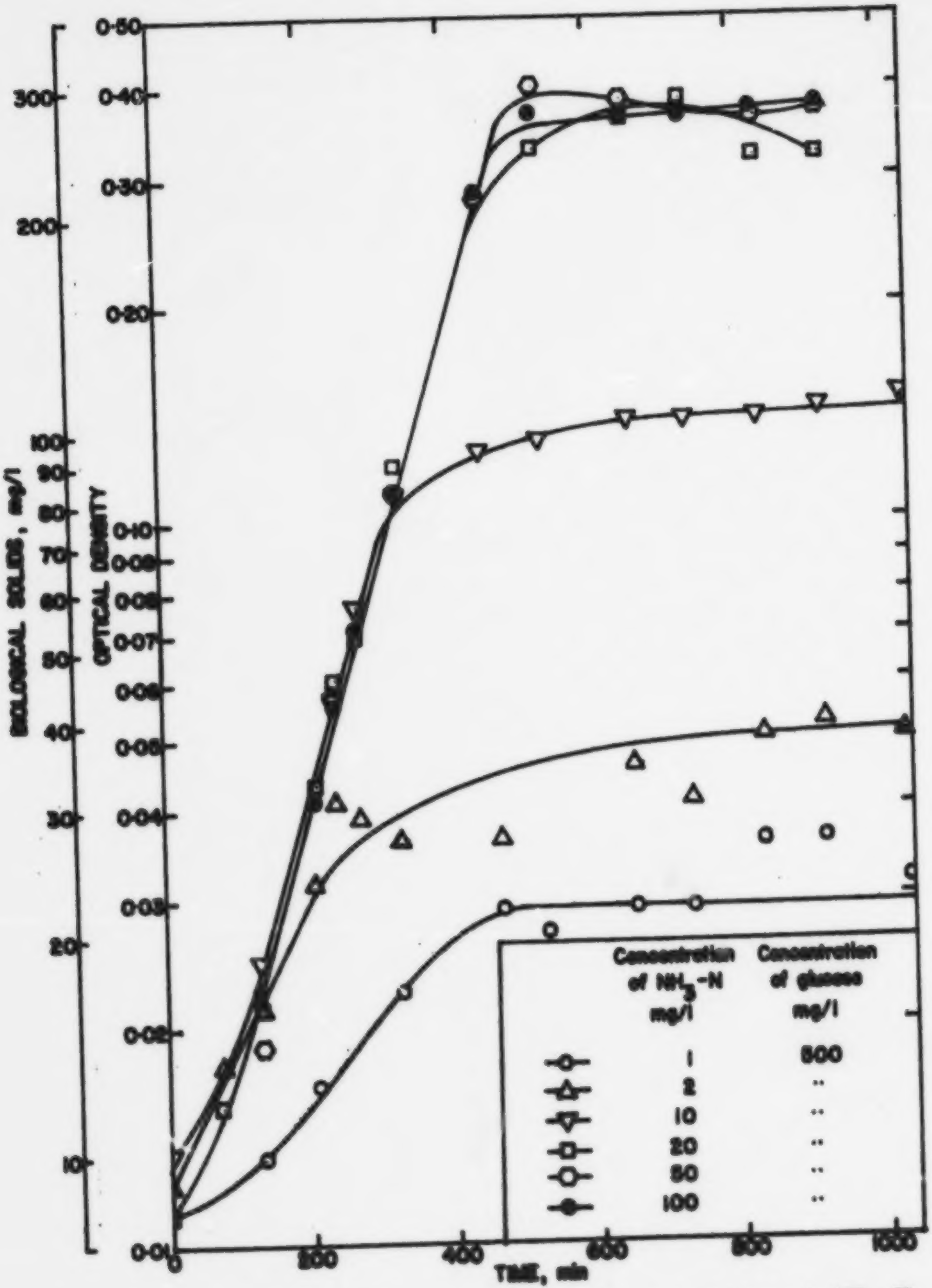

Figure 1 - Growth on glucose at various levels of NH$_3$-N. Cells of sewage origin from chemostat operating on glucose at a COD/N ratio = 40/1 and D = 1/2 hr^{-1}.

seen, in all experiments, that for the level of carbon source employed (500 mg/l glucose) essentially all of the NH_3-N was assimilated when the amount added was 20 mg/l. However, in nearly all cases the value of μ had attained (or approached) a maximum at this level. This finding suggests that the external concentration of NH_3-N did not limit μ, and, if any theoretical significance can be assigned to any relationship which might be shown between μ and NH_3-N concentration, it should not be based on decreased concentration gradient (or driving force) between the extracellular and intracellular boundaries. In an attempt to gain an insight into possible relationships between μ and NH_3-N concentration, values of μ were plotted against the various nitrogen levels employed. Examples of such plots for data taken from Table I are given in Figures 2 (for cells originally grown at a COD/N ratio of 40/1), and 3 (for cells originally grown at a COD/N ratio of 25/1). Curves through the data were drawn, in general, in accordance with the hyperbolic function of Monod. However, it is seen that the data were too scattered and there were too few plotting points to conclude with any degree of surety that the hyperbolic function provided a usable fit. Indeed, the linear discontinuous function suggested by Garrett and Sawyer for carbon source-limited systems (5) could have been employed. In previous work on carbon-limited systems (2)(3) it has been shown that when the value of the saturation constant, K_s, is low in relation to the concentration of growth-limiting nutrient needed to approach μ_m, the hyperbolic function rises so rapidly from the origin that the linear discontinuous function can, for all practical purposes, be employed. The K_s values obtained from the data plotted in Figures 2 and 3 are, in general, rather low with respect to concentrations needed for μ_m when compared with K_s values obtained when carbon source is the limiting nutrient (2)(3).

It has previously been shown in the present report (see Table I) that μ_m values were attained in general between NH_3-N concentrations of 10 and 20 mg/l. Therefore, the most informative data for defining a relationship between μ and NH_3-N concentration lies below 20 mg/l NH_3-N levels. For the results presented thus far, there were only 2-3 plotting points available in this range. For this reason, additional experimentation was conducted using heterogeneous populations developed (on glucose) from sewage seed obtained from the municipal treatment plant at Stillwater, Oklahoma. A plot of specific growth rate (on 500 mg/l glucose) vs. NH_3-N in the range of 2 to 20 mg/l is shown in Figure 4. The maximum growth rate was, as in most of the previous cases, attained or closely approached before the 20 mg/l level and the value for K_s is in general agreement with the values found previously. The value of μ_m was slightly higher than the values observed previously.

Concerning the shape of the relationship between μ and NH_3-N concentration, it may be discerned that a straight line drawn through the origin intersecting a horizontal line at μ_m (the discontinuous linear function) would not be as satisfactory as the curve shown.

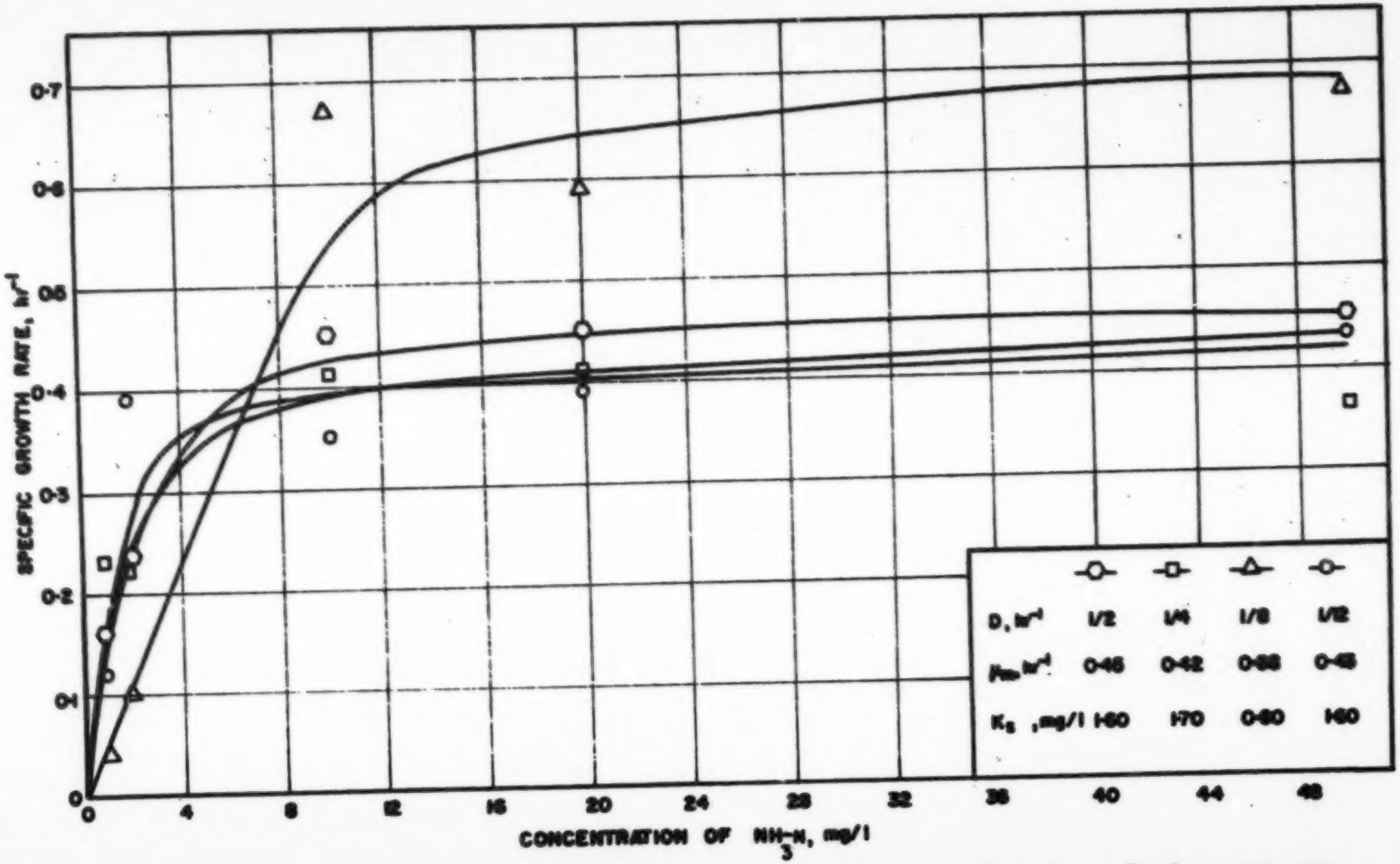

Figure 2 - Effect of NH_3-N concentration on specific growth rate. Carbon source, glucose; cells of sewage origin from chemostat operating on glucose at a COD/N ratio = 40/1 at various dilution rates.

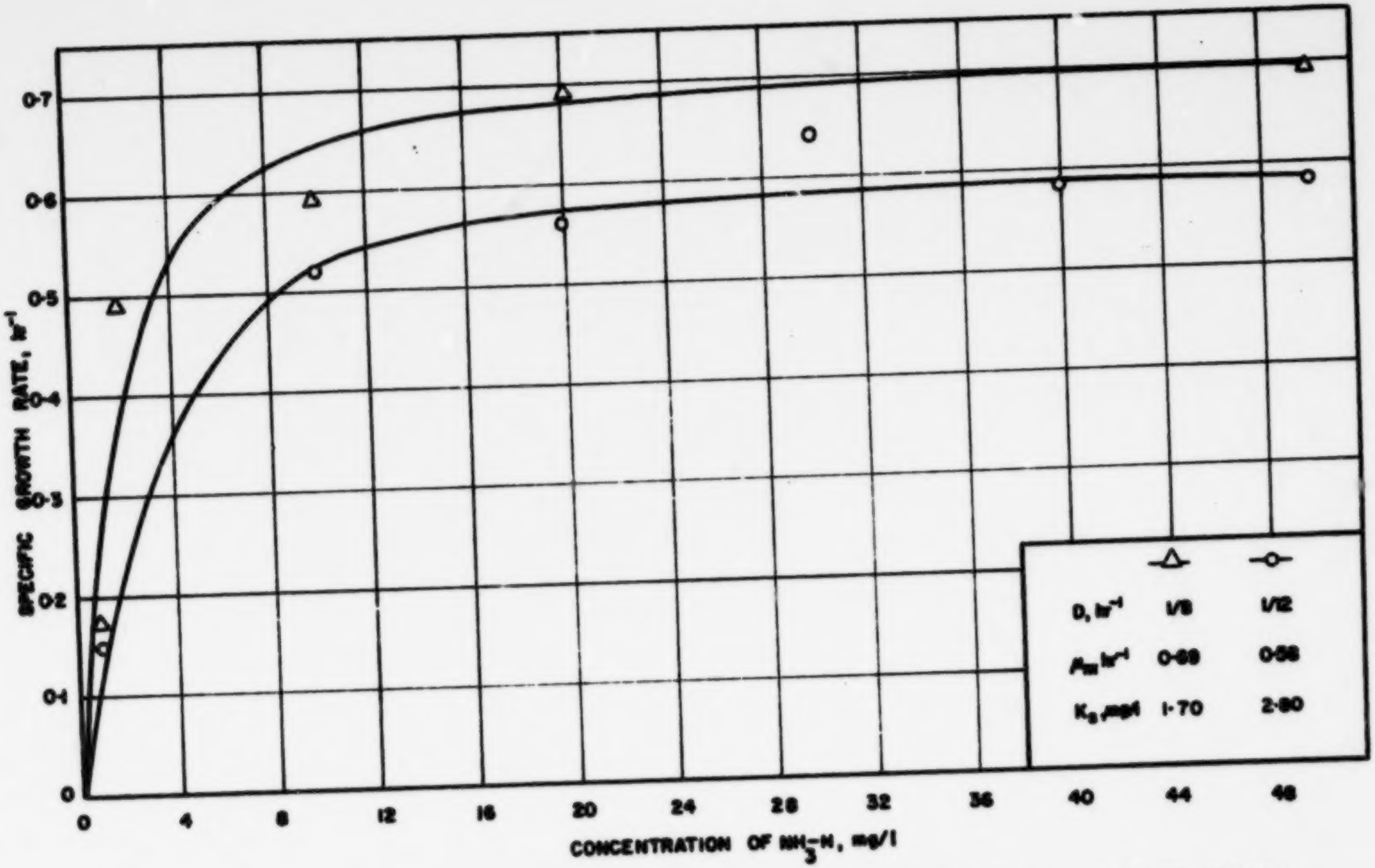

Figure 3 - Effect of NH$_3$-N concentration on specific growth rate. Carbon source, glucose; cells of sewage origin from chemostat operating on glucose at a COD/N ratio = 25/1 at various dilution rates.

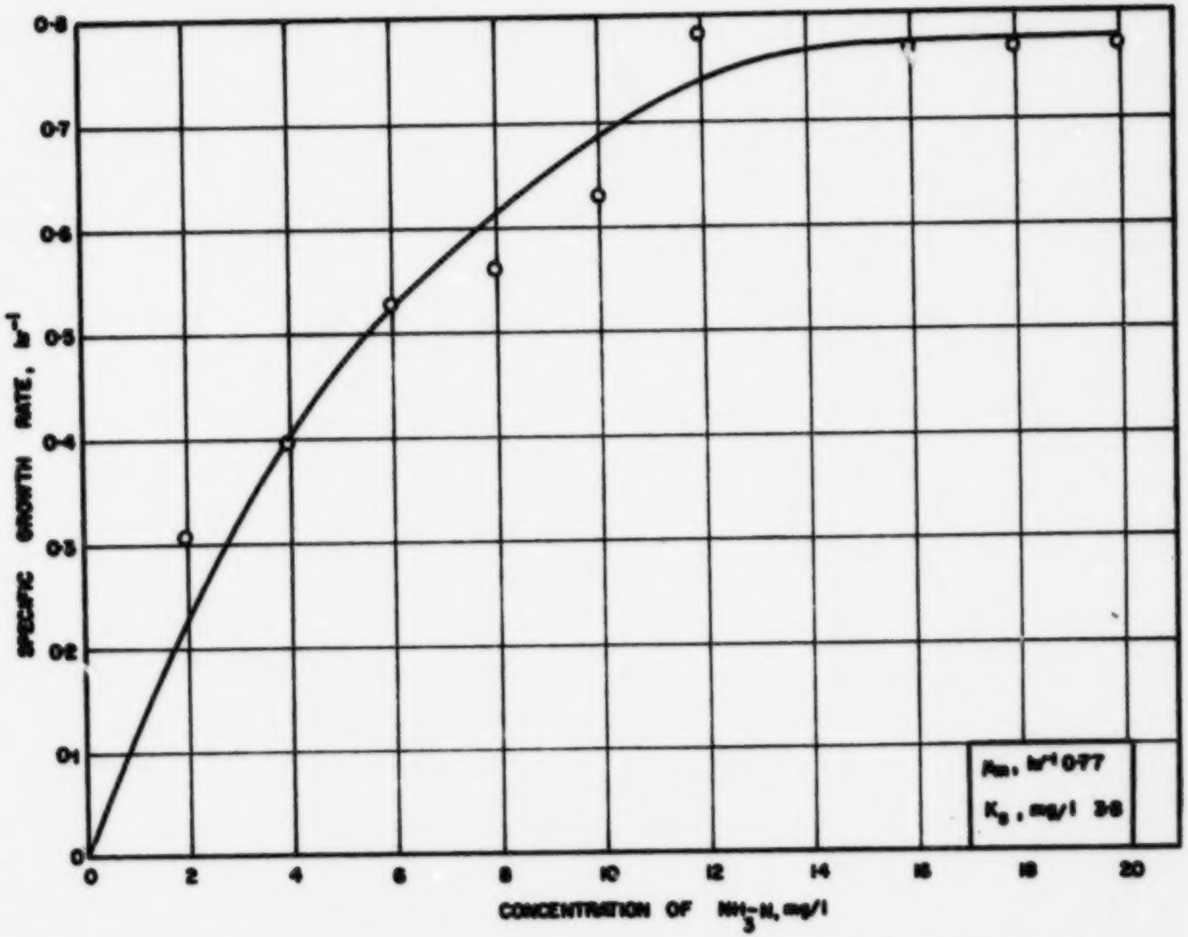

Figure 4 - Effect of NH_3-N concentration on specific growth rate. Carbon source, glucose; cells of sewage origin.

From the present results it would appear that for heterogeneous microbial populations an empirical relationship of the type proposed by Monod (hyperbolic) depicting μ as a function of the concentration of growth-limiting nutrient for systems limited by the concentration of carbon source can be used for depicting μ as a function of the concentration of nitrogen source when the nitrogen source is the growth-limiting nutrient. The value of μ_m in these studies (as in others wherein carbon source was limiting nutrient (2)(3)) was not a constant. In general, it ranged between 0.4 and 0.7 hr^{-1}, values which compare fairly well with those observed for heterogeneous populations in carbon-limited systems (2)(3). Since μ_m as well as K_s values differ for various organisms, it must be expected that these "constants" will vary for heterogeneous populations since changes in microbial predominance occur from time to time regardless of the constancy of operational conditions (6). It may be discerned from Table I that the past growth history of the cells exerted no definable pattern on the variation in μ_m. The same may be said for K_s (see Figures 2, 3, and 4).

From an engineering standpoint it is fortunate that the range of values for these constants is as narrow as it is for a specific operational condition. In general, the K_s values for nitrogen-limited systems grown on an easily metabolized carbohydrate (glucose) ranged between 1.5 and 4 mg/l, compared to K_s values in the general range of 75-125 mg/l for carbon-limited systems run using the same operating conditions (2)(3).

The values of K_s for nitrogen-limited systems has also been found to be much lower than for carbon-limited systems in studies using pure cultures (4). The concentration of NH_3-N needed to permit the specific growth rate to approach μ_m is estimated (see Figures 2, 3, and 4) to be approximately 15 mg/l. In comparable carbon source limited systems, the concentration of carbon source needed to permit the specific growth rate to approach μ_m was approximately 500 mg/l. The measured COD of 500 mg/l glucose is approximately 530 mg/l and the COD/N ratio for attainment of μ_m in a system limited by neither carbon source or nitrogen source may then be estimated roughly as the ratio 530/15 = 35/1. The BOD of this concentration of glucose is approximately 370 mg/l (i.e., 0.7 x COD) and the ratio of BOD/N = 25/1. These values agree rather well with the conclusions concerning optimum COD/N and BOD/N ratios based upon the studies performed in the completely mixed continuous flow reactors (1) and the BOD/N ratio is only slightly higher than that routinely employed in biological treatment based upon the recommendation of Sawyer and his co-workers (9). However, it should be noted that it is known from the continuous flow reactor studies (1) that ideal or optimum carbon source/nitrogen ratios should not be based solely on the ratio found for "balanced" growth, but should include consideration of the reactor detention time and of leakage of nitrogen in the effluent. It is also known that continuous biological removal of a carbon source and concomitant biological solids production can be made to occur in the absence of a nitrogen source, i.e., under conditions of oxidative assimilation (7)(8). This is possible, provided a portion of the settled biological solids is subsequently subjected to aeration under endogenous conditions, with respect to carbon source, in the presence of an exogenous source of nitrogen. Under such conditions of operation, excellent COD removal efficiency has been demonstrated, using comparable COD/N ratios as high as 70/1 and a reactor detention time as low as four hours.

ACKNOWLEDGEMENT

This work was supported by a Research Grant WP 00325 from the Federal Water Pollution Control Administration, U. S. Department of the Interior.

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PAPER 20

Isaacs, W. P., and Gaudy, A. F. Jr., "Comparison of BOD Exertion in a Simulated Stream and in Standard BOD Bottles." Proceedings, 22nd Purdue Industrial Waste Conference, Purdue University, May, 1967.

COMPARISON OF BOD EXERTION IN A SIMULATED STREAM AND IN STANDARD BOD BOTTLES

By

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Today the measurement of changes in dissolved oxygen concentration occurring in a polluted system can be accomplished quite accurately; however, the intricate biological kinetics which determine the course of deoxygenation are at present only partially explained. First order reaction kinetics have been applied almost exclusively in the past to describe the exertion of BOD in receiving streams. The standard BOD bottle test has been used to define biological deoxygenation regardless of the particular process or system being considered, although the technique was originally intended for use in predicting the course of oxygen utilization and oxygen recovery in receiving

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Presented at the 22nd Industrial Waste Conference, Purdue University, Lafayette, Indiana, May 2-4, 1967.

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streams; i.e., in relatively dilute systems. Its use in conjunction with the reaeration equation has been accepted for many years to predict the oxygen sag curve in receiving streams. In using the sag equation, the deoxygenation constant, k_1, determined in the BOD bottle, is assumed to be equal to the value of k_1 in the receiving stream. Most often the reaeration constant, k_2, is determined indirectly by substituting the value of k_1, determined using the BOD bottle technique, and other parameters into the sag equation and solving for k_2. In general, it may be said that the applicability of the sag equation has not been experimentally proven for the conditions assumed in its development, and there are many questionable aspects concerning its applicability. For example, the effect of turbulence and mixing upon metabolic activity has not been precisely defined, and there is no certainty that the k_1 value obtained in the BOD bottle is valid for the receiving stream or for a turbulent system. The effect of turbulence and mixing on biological activity has received some investigational attention (1, 2); however, the complexity of the problem has made separation of the variables involved quite difficult. Furthermore, the effect of concentration of biological solids or initial seeding concentration on deoxygenation kinetics has been only partially delineated with respect to kinetic order and kinetic discontinuities which may appear in the BOD exertion curve (3, 4). Also, in natural streams bacterial deoxygenation and physical reaeration are not the only factors which contribute to the oxygen balance. Photosynthesis and algal respiration, as well as benthal demands may play an important but as yet relatively unexplored role in determining the course of the dissolved oxygen sag for any particular reach of stream. It would appear that controlled experimentation in which each of the contributing mechanisms can be separately studied will almost certainly be required before the most useful equation(s) of predictive value can be devised.

During the course of an investigation made by the authors to determine factors affecting the reaeration velocity constant, k_2, in a simulated river (5) there was an opportunity to determine whether k_1 values obtained using the standard BOD bottle technique were equivalent to k_1 values determined in the simulated river when it was subjected to a known concentration of organic pollution. Gates, et al. (2) have presented results of laboratory studies which cast serious doubt upon the applicability of the sag equation in systems subjected to concurrent physical aeration and biological deoxygenation. However, in their studies no attempt was made to separate the individual effects of reaeration and deoxygenation, nor was there an attempt to predict equivalence of k_1 in the aerated and in the quiescent system (BOD bottle). The present study was designed specifically to gain an insight into the equivalence of k_1 determined in the BOD bottle and in a system subjected to the type of physical aeration (surface aeration) existing in a flowing body of water.

EXPERIMENTAL APPROACH

The kinetic course of changes in dissolved oxygen for a system in which oxygen is replenished by means of atmospheric reaeration and oxygen is depleted by biological metabolism has been interpreted almost exclusively in the past by the classical "sag equation"

$$D_t = \frac{k_1 L_o}{k_2 - k_1}\left[10^{-k_1 t} - 10^{-k_2 t}\right] + D_o 10^{-k_2 t} \qquad (1)$$

This equation is the integrated solution of the following basic differential equation

$$\frac{dD}{dt} = \frac{dD_1}{dt} - \frac{dD_2}{dt} \qquad (2)$$

where $\frac{dD}{dt}$ represents the change in dissolved oxygen deficit due to the combined effects of reaeration $\left[\frac{dD_2}{dt}\right]$ and deoxygenation $\left[\frac{dD_1}{dt}\right]$. The two processes are assumed to obey first order reaction kinetics, i.e.,

$$\frac{dD_1}{dt} = 2.303\ k_1 L \qquad (3)$$

and

$$\frac{dD_2}{dt} = -2.303 k_2 D \qquad (4)$$

Making these assumptions, equation (2) can be solved to yield equation (1), the classical sag equation introduced by Streeter and Phelps (6).

Since the validity of equation (3), i.e., first order decreasing rate kinetics for biological deoxygenation, is quite controversial, the authors sought to make an independent determination of biological deoxygenation in an aerating system using the following expression:

$$\Delta D = \Delta D_1 + \Delta D_2 \tag{5}$$

where ΔD (the change in deficit) can be directly measured as the observed change in dissolved oxygen deficit within the reactor over a short time interval, Δt. For a controlled system where the reaeration rate, k_2, is known for the experimental conditions, ΔD_2 can be computed in accordance with first order reaction kinetics for the reaeration process:

$$\Delta D_2 = -2.303\ k_2 \bar{D}\ \Delta t \tag{6}$$

where $\bar{D}$ is the average deficit occurring during the small increment of time, Δt. Equation (5) can be solved for ΔD_1, the change in dissolved oxygen deficit due to biological deoxygenation:

$$\Delta D_1 = \Delta D + 2.303 k_2 \bar{D}\ \Delta t \tag{7}$$

Equation (7) can be integrated numerically for "n" periods of time, Δt, to yield the oxygen uptake response due to biological deoxygenation, since ΔD_1 is equivalent to $\Delta(DO)_1$ or the incremental change in O_2 uptake.

$$\sum_{i=0}^{n} \Delta D_{1,i} = \sum_{i=0}^{n} \Delta D_i + \sum_{i=0}^{n} 2.303 k_2 \bar{D}_i (\Delta t)_i \tag{8}$$

The parameter $\sum_{i=0}^{n} \Delta D_{1,i}$ can be plotted vs. time to yield the oxygen uptake curve for the biological deoxygenation process. It was the purpose of these investigations to compare the resulting oxygen uptake curve obtained in the aerating system with the oxygen uptake or BOD curve determined using the BOD bottle technique.

Experimental Apparatus

The experimental apparatus utilized in this investigation is a somewhat unique laboratory device developed by the authors. The flow conditions which can be made to prevail in this experimental apparatus closely simulate the flow conditions which prevail in a geometrically uniform open stream channel. Two views of the apparatus are shown in Figure 1; also shown is the apparatus used to hold and locate the velocity meter at any desired channel cross-section. The tank is a right circular cylindrical torus. Placed inside the tank are two right circular cylindrical shell walls which are driven by variable speed motors. The movement of these walls (or simulated channel or river banks) imparts velocity to the water and permits attainment of a velocity profile representing a desired experimental stream condition. The tank has a maximum inside wall height of twenty-two inches, and a clear spacing between the moving walls of twelve inches. The movable walls are placed 0.125 inches inside the fixed walls of the torus-shaped tank. The diameter of the inside rotating cylindrical shell is four feet, and the diameter of the outside rotating cylindrical shell is six feet. For the experiments reported herein, an eighteen-inch water depth was used. The volume of water in the system was approximately 23.6 cu. ft., or 668 liters.

Experimental Protocol

The following general experimental procedure was used in conducting all of the experiments herein reported. The simulated stream apparatus was filled with tap water to desired volume, adjusted to ambient temperature, and was allowed to equilibrate overnight. On the following morning the apparatus was started up, and the water was brought to the desired stream velocity. The velocity of flow in any stream cross-section was determined by measuring the velocity at 25 points spaced approximately at equal distance throughout a cross-section. An Ott-meter (propeller-type velocity meter) was used to determine point velocities. The arithmetic average of the 25 point velocities was taken as the average velocity over the cross-section. Cross-sectional velocities so measured around any portion of the torus were identical to those measured at any other cross-section.

After determination that the velocities attained were those intended for a particular experiment, the dissolved oxygen of the water was removed by sodium sulfite at a dosage sufficient to remove 8 mg/1 DO. Cobalt chloride was added as a catalyst at a dosage of 0.02 mg/1* (7). The system was allowed to equilibrate, and when there was a slight but significant amount of dissolved oxygen present, the zero time was established for a reaeration experiment to determine k_2. Samples were taken in order to establish the kinetic course of reaeration and the saturation value, C_s.

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*A concentration of 10 mg/1 of Co is reported to be required to halve the maximum growth of Neurospora crassa in a normal basal media (8).

Upon completion of the reaeration experiment, a small amount of tap water was added to replace the volume taken for samples during the reaeration experiment. Inorganic constituents of standard BOD dilution water were added in accordance with concentrations recommended in Standard Methods for the Examination of Water and Wastewater (9). The simulated stream was then allowed to run overnight at the same velocity employed in the reaeration experiment. On the following morning another check on the velocity was made, and a measured amount of substrate was placed into the system. Settled sewage seed from the Stillwater municipal treatment plant (aged 24 to 48 hours) was placed into the system at a dosage of 5 ml/1. The system was then allowed to equilibrate for thirty minutes, and dissolved oxygen concentration was measured in order to establish the DO level at the initiation of a dissolved oxygen sag experiment. Also, at this time samples of the water were removed from the apparatus and placed in BOD bottles (1/5 dilution). Dissolved oxygen was determined on these samples at appropriate time intervals. The BOD bottles were incubated at 23°C. and the temperature in the simulated stream apparatus during the period of this investigation was $23^\circ \pm 1^\circ$C. It was necessary to dilute the "river water" 1/5 in order that the dissolved oxygen in the BOD bottles would not be depleted during the determination of BOD exertion. Although this procedure is in accordance with general practice for conducting BOD determinations on natural stream waters, it is important to note that in addition to providing a 1/5 dilution of organic matter (substrate), it also provided a 1/5 dilution of seeding material, i.e., the amount of initial seed in the BOD bottle was 1/5th the amount of initial seed in the simulated stream. In order to delineate the effect that this procedure might have on comparative analysis of the course of oxygen uptake in the BOD bottle and in the turbulent system, a separate BOD bottle experiment was run in which the concentration of initial seed was the same as that which was employed in the simulated stream.

Analyses

BOD determinations were run in accordance with Standard Methods for the Examination of Water and Wastewater; dissolved oxygen was determined by the Alsterberg (azide) modification of the Winkler method. The membrane filter technique was employed for determination of biological solids concentration (pore size, 0.45μ). Substrate concentration (glucose) in the turbulent system was determined using the anthrone technique (10).

RESULTS

A synthetic waste consisting of equal parts of glucose and glutamic acid was employed, since such a mixture has been reported to have a reasonably stable oxidation rate similar to that obtained with many municipal wastes (k_1 = 0.16 - 0.19)(9). For the experiments herein reported, 22.0 mg/l glucose and 22.0 mg/l glutamic acid were used as a substrate. Figures 2 and 3 show the results of two experiments which were accomplished under essentially identical conditions. Shown on the figures are the following curves:

a) The reaeration curve for the simulated stream, determined prior to introducing organic matter. The values plotted are those obtained using the reaeration constant, k_2, and the dissolved oxygen saturation value, C_s, determined during the reaeration experiment which immediately preceded the introduction of the organic pollution.

b) The observed dissolved oxygen concentration or DO sag curve in the simulated river.

c) The oxygen uptake curve for the turbulent system computed from the oxygen sag curve data and the reaeration curve data, and the oxygen uptake curve obtained in the quiescent BOD bottle system.

d) Also shown (on Figure 2) is the theoretical sag curve based upon the k_1 and L_o values determined from the BOD bottle system assuming first order kinetics.

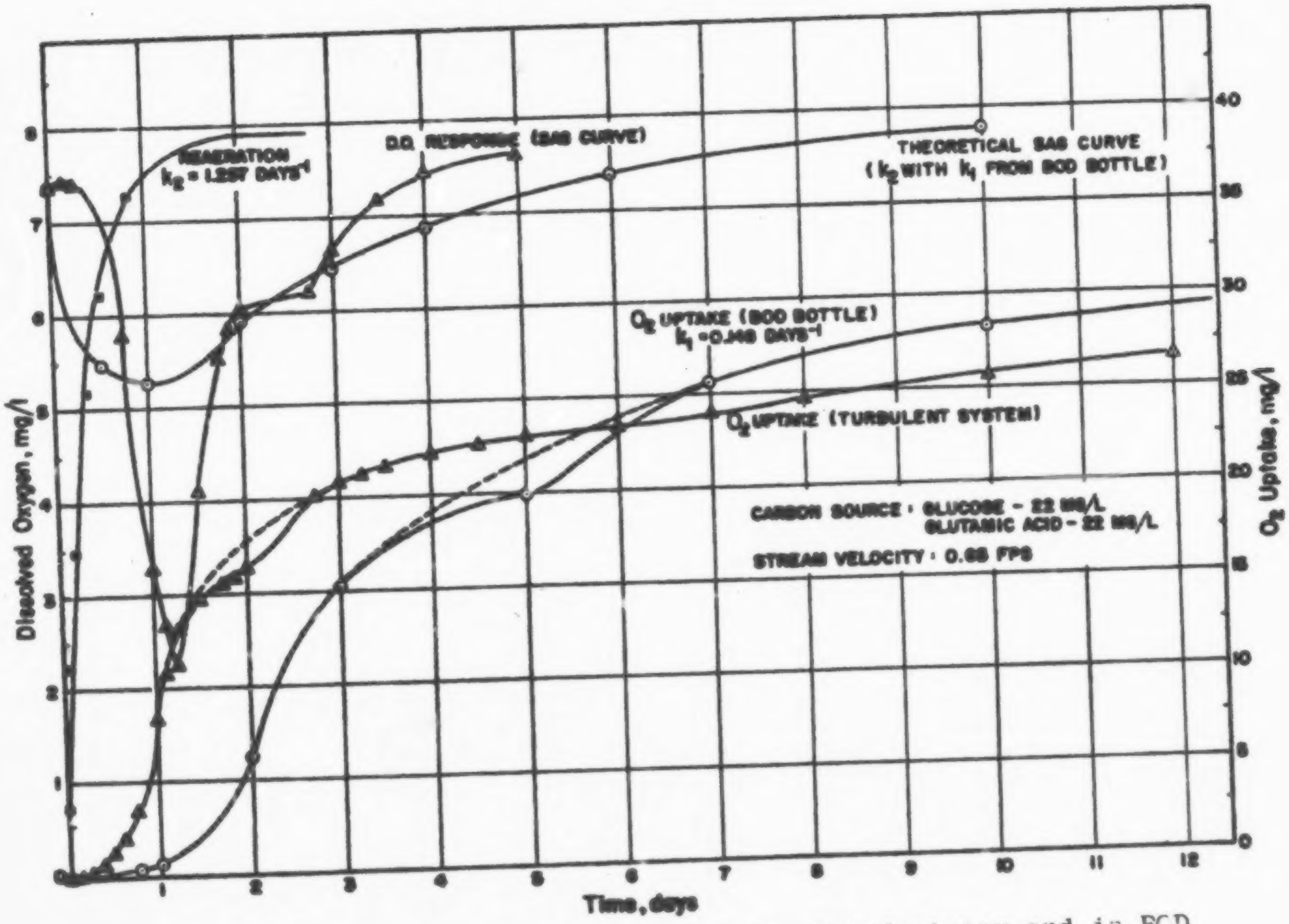

Fig. 2. Comparison of oxygen uptake in a simulated stream and in BCD bottles - Experiment No. 1.

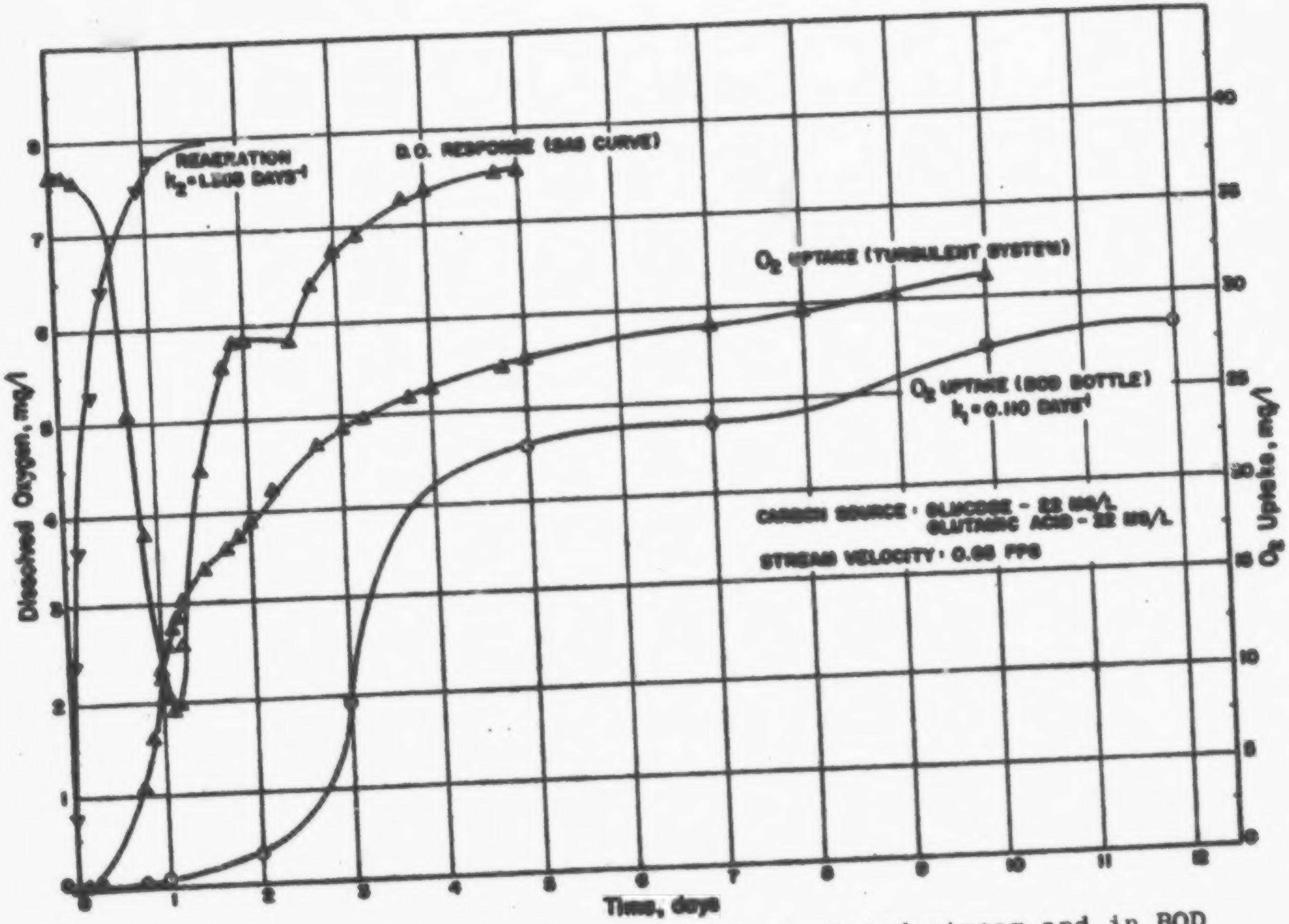

Fig. 3. Comparison of oxygen uptake in a simulated stream and in BOD bottles - Experiment No. 2.

It can be seen that after an initial lag (1/2 day to 3/4 day) the simulated river (turbulent) system exhibited a very high oxygen demand (see sag curve). After a minimum value of dissolved oxygen was reached, there was a rapid DO recovery period in which the sag curve was nearly parallel to the reaeration curve for approximately 0.75 day. It can be seen by comparing this leg of the sag curve and the oxygen uptake curve for the turbulent system that the rapid recovery in dissolved oxygen occurred during the "plateau" period of the oxygen uptake curve. After the period of initial recovery in the sag curve, there was a second strong oxygen demand which existed for approximately 0.75 day, followed by a fairly sharp recovery. However, the shape of the second recovery curve when compared to the reaeration curve computed from the value of k_2 indicates that some small oxygen demands were still being exerted in the system. It is also readily observed that the theoretical sag curve computed from the known k_2 value and the value of k_1 observed in the BOD bottle studies provided virtually no prediction of the observed sag curve in the simulated river system.

Comparing the oxygen uptake curves in the BOD bottle and in the turbulent system, one can readily observe some similarities. The basic shape of the curves is similar; both contain a "plateau", yielding diphasic oxygen uptake. However, there are differences in the amount of oxygen uptake in each stage. One may discern from the oxygen uptake curves that there occur, in order , a lag of variable proportions for the BOD bottle and turbulent system, a fairly rapid first stage of oxygen uptake, a plateau region, and a relatively rapid second stage of oxygen uptake which tails off to a very slowly increasing oxygen uptake curve. This last, very slow, stage of oxygen uptake may be attributed to endogenous respiration, and the difference in the initial lag periods may be attributed to the difference in initial seed concentrations in the turbulent and BOD bottle systems.

In order to gain additional insight into this apparent three-phase BOD exertion, a third experiment was conducted under the same conditions as the previous two. However, in this experiment the course of carbohydrate removal was measured using the anthrone method (10) and biological solids were measured using the Millipore filter technique, as shown in Figure 4. The DO response curve and the oxygen uptake curves for the turbulent and quiescent systems are very similar to those obtained for the first two experiments. It can be seen in Figure 4 that carbohydrate removal parallels the rapid first stage BOD exertion. This is discernible by comparison of the DO response curve and the carbohydrate removal curve with the oxygen uptake curve for the turbulent system. The point of complete removal of carbohydrate occurs at the same time as the point of minimum dissolved oxygen. Furthermore, biological solids increased during the first stage of oxygen exertion, and peaked concurrently with the minimum DO response and the point of complete removal of carbohydrate. Biological solids concentration remained constant for approximately six hours, i.e., during the plateau period, and then dropped off rapidly during the second stage of oxygen demand.

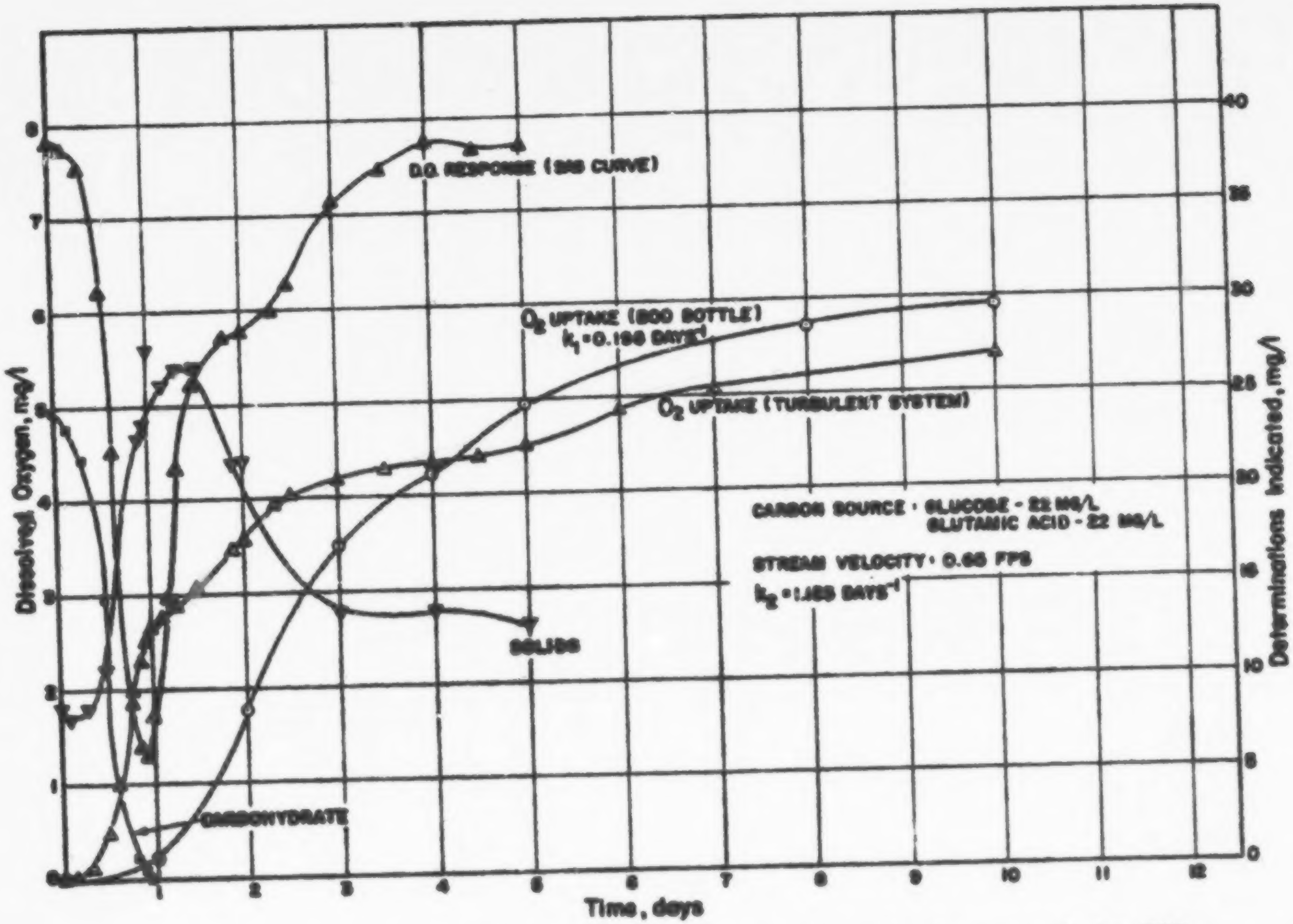

Fig. 4. Comparison of oxygen uptake in a simulated stream and in BOD bottles - Experiment No. 3.

After a sharp drop in biological solids during the second stage O_2 demand, the solids concentration gradually decreased during the third stage of oxygen uptake.

Although protozoan counts were not conducted during this experiment, microscopic examinations made during the period of second stage oxygen exertion showed that significant numbers of protozoa were present. Distinct clarification in the turbulent system was always observed during the second stage oxygen uptake.

It was of interest to gain some insight into the effect that increased velocity (and thus increased k_2) would have on the course of oxygen uptake. Figure 5 shows the results of an experiment for which the reaeration constant was approximately twice that of the previous experiments. The same general trends were observed, although in this experiment the first stage oxygen demand was smaller and the second stage demand larger than that observed for the previous experiments. However, the total oxygen demand was approximately the same. Oxygen uptake curves for the turbulent systems for all four experiments are given in Figure 6.

Experiments were also conducted using effluent from the primary clarifier of the municipal sewage treatment plant in Stillwater, Oklahoma. Figure 7 shows the results obtained using a 1/5 dilution of settled sewage. As in the previous experiments, there is evidence in the turbulent system for phasic oxygen uptake; however, the third, or endogenous phase was significantly higher for the sewage system than for the glucose-glutamic acid systems. Sufficient BOD bottle samples

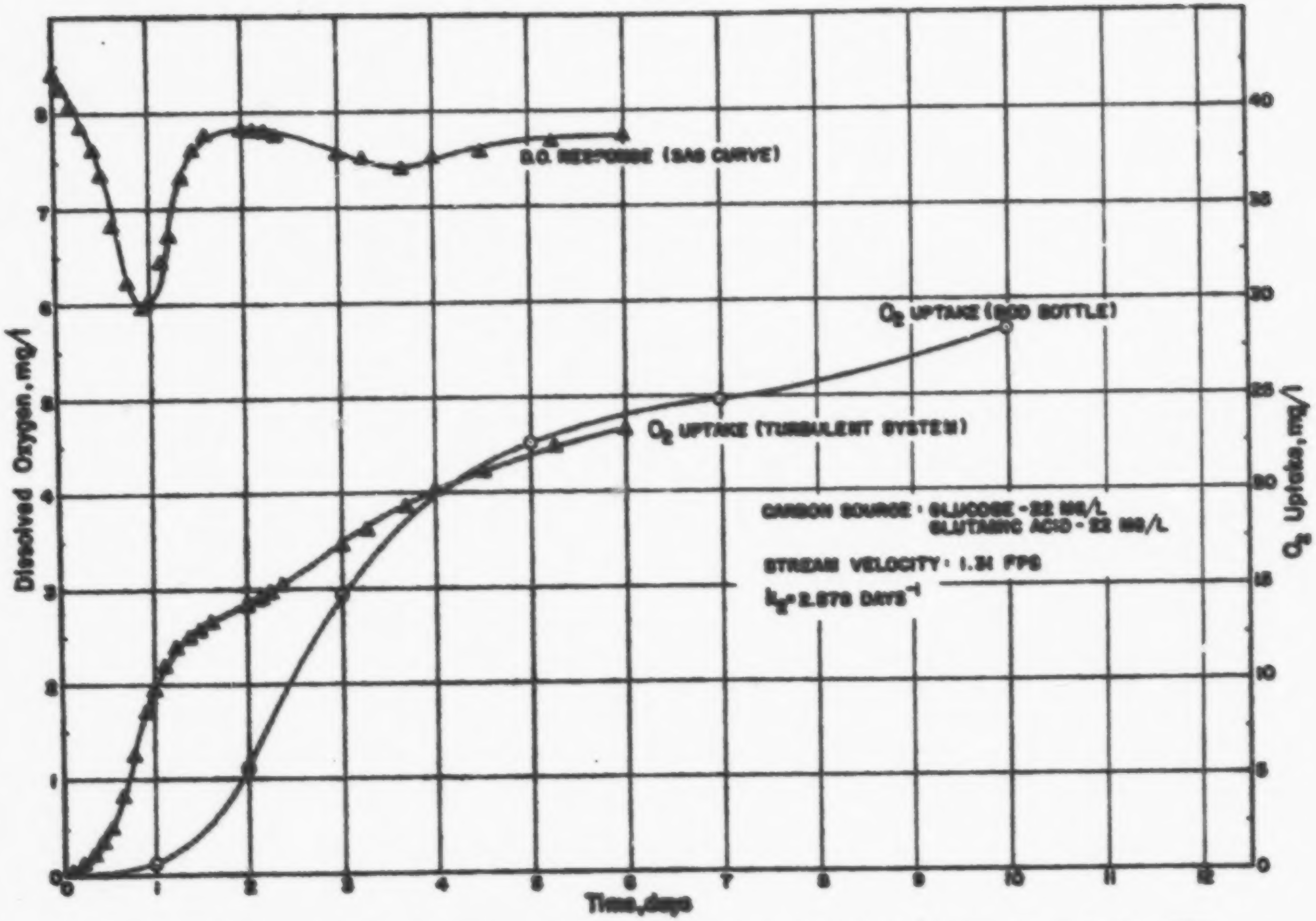

Fig. 5. Comparison of oxygen uptake in a simulated stream and in BOD bottles - Experiment No. 4.

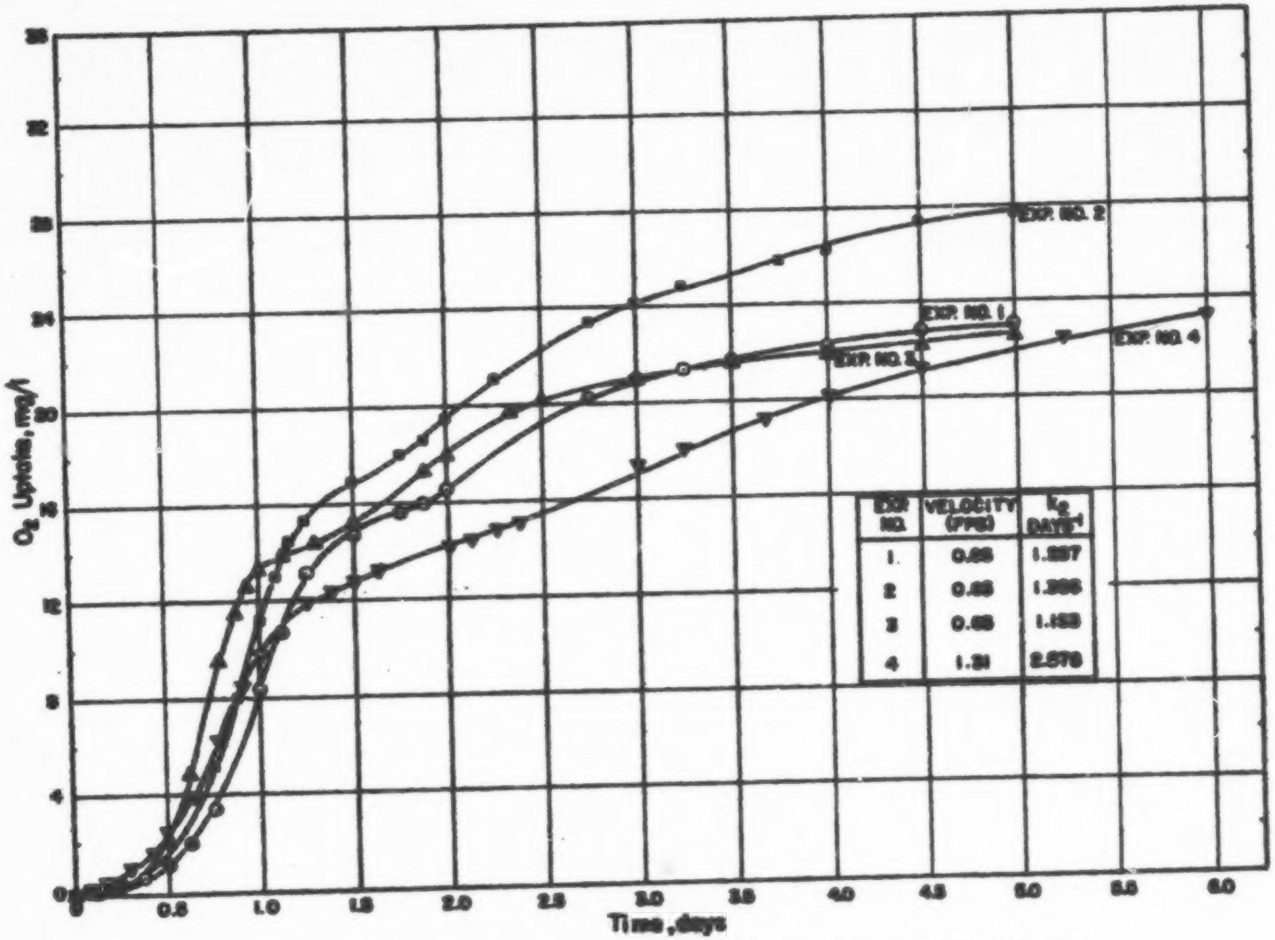

Fig. 6. Measurements of oxygen uptake in a simulated stream - Experiments 1 through 4.

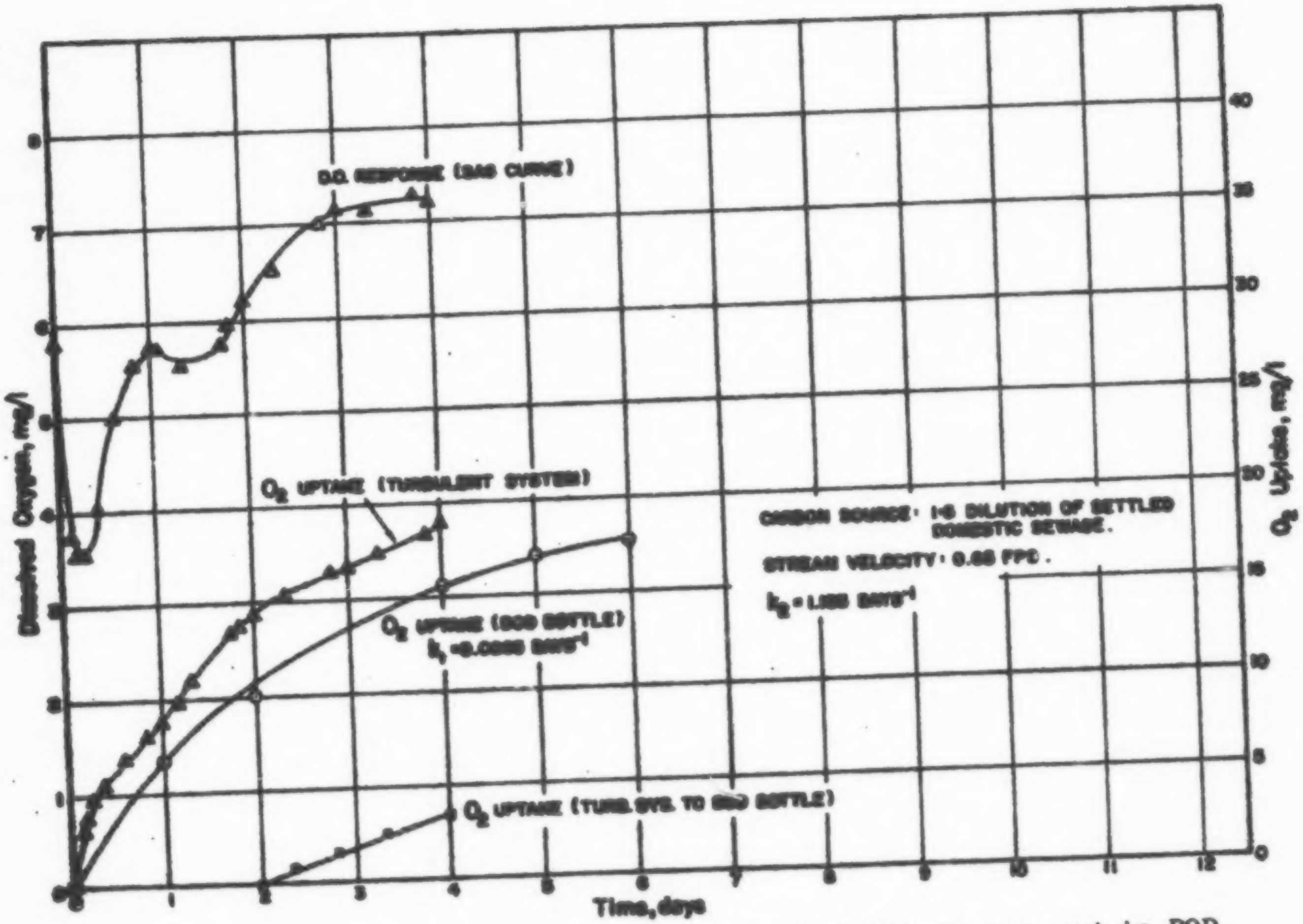

Fig. 7. Comparison of oxygen uptake in a simulated stream and in BOD bottles - Experiment No. 5.

were not taken to detect any phasic tendency of oxygen uptake in the BOD bottle system. The lower curve on Figure 7 shows oxygen uptake for samples transferred from the turbulent system to BOD bottles on the second day of the experiment. Comparison of these data with the oxygen uptake values calculated from the turbulent system indicates that the curves follow a parallel course.

An experiment was also conducted in which glucose (44 mg/1) was the sole source of carbon. The results of this experiment are shown in Figure 8. Sampling was not sufficiently frequent in the BOD bottle system to detect any discernible phasic tendency in exertion of BOD. Also, in the turbulent system where many samples were obtained, the phasic tendency was not as apparent as it was in any of the previous experiments.

One of the primary differences between oxygen uptake in the BOD bottles and in the turbulent system was the presence of a rather pronounced lag in the BOD bottle system when compared to the oxygen uptake curve obtained in the simulated stream. The two major differences between the systems were the presence of turbulence or mixing energy in the simulated stream and the higher initial seed concentration in this system. In order to test the significance of initial seed concentration in the BOD bottle, an experiment was set up in which 5 ml/1 of settled sewage seed (the same concentration as was used in all of the turbulent systems except those experiments using settled sewage seed as substrate) was used as the seed concentration for all dilutions in the BOD bottle. Three dilutions (1/1, 1/2, 1/5) of substrate (44 mg/1 glucose-glutamic acid) were examined.

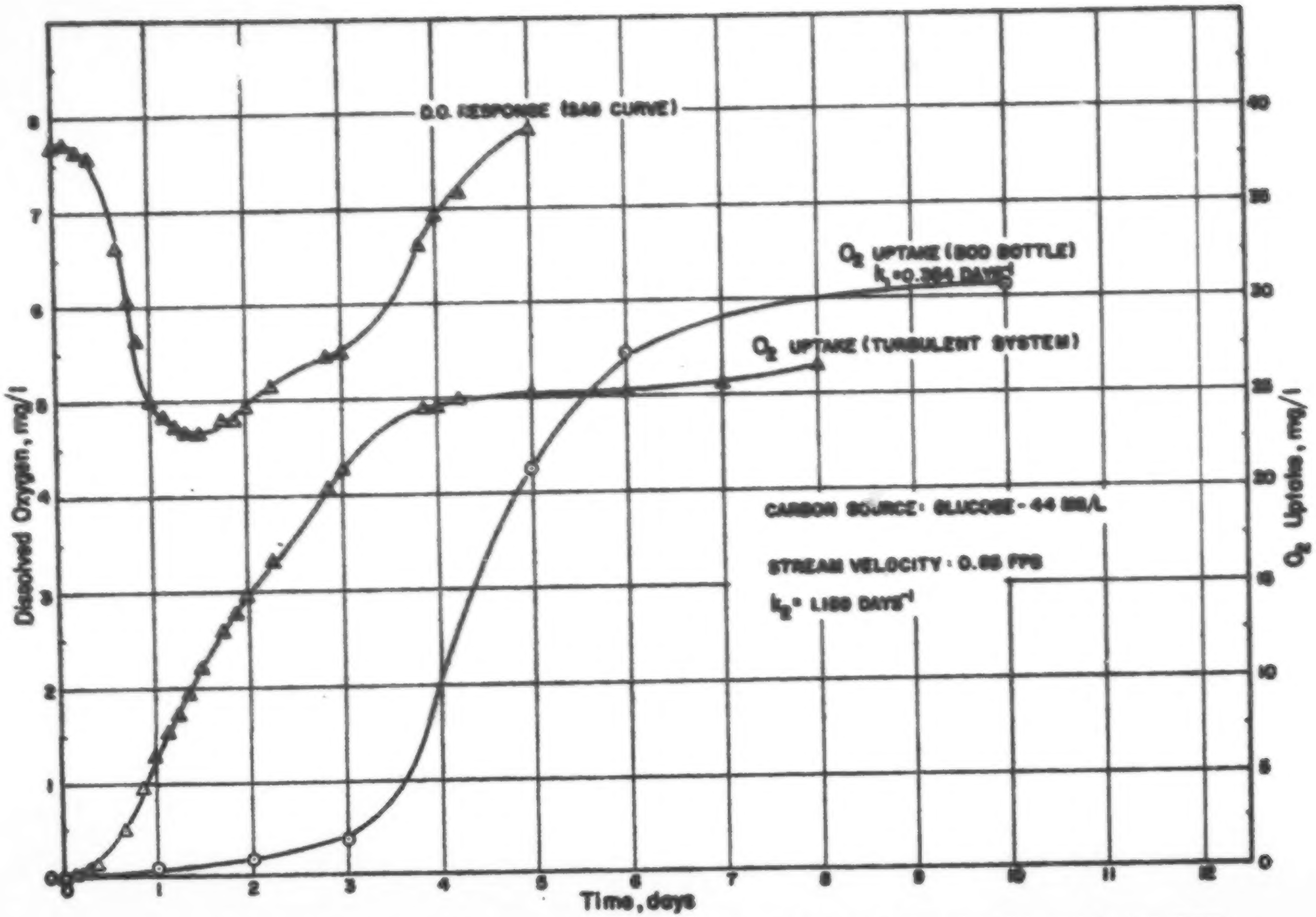

Fig. 8. Comparison of oxygen uptake in a simulated stream and in BOD bottles - Experiment No. 6.

Results of the experiment are shown in Figure 9. It is seen that there was no discernible lag in oxygen uptake, and at the 1/2 day sample, oxygen uptake in the 1/1 dilution was lower than in the other two. For the lower dilution, dissolved oxygen was completely exhausted within one day. It is also noted that the oxygen uptake for the 1/2 dilution yielded BOD values slightly less than the 1/5 dilution. When the oxygen uptake values obtained in this experiment for the 1/5 dilution were compared with the oxygen uptake curves obtained in the turbulent systems for which the substrate concentration and the seed dilution were the same as in this experiment, the oxygen uptake was at all times slightly greater than that exerted in any of the equivalent turbulent systems (see Figure 10). Multiphasic oxygen uptake was not discernible for this BOD bottle experiment, although the infrequency of sampling may not have allowed for its detection.

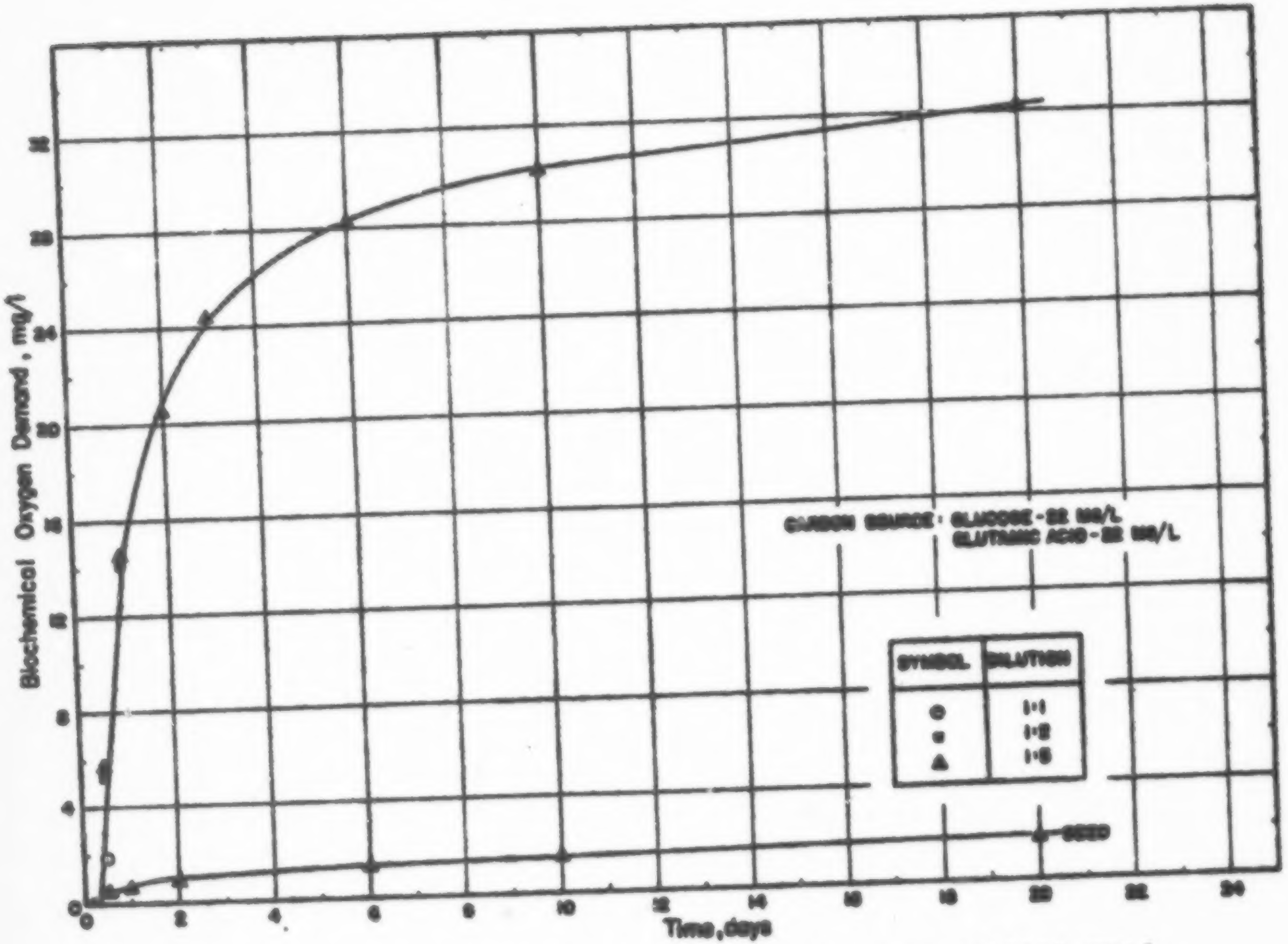

Fig. 9. Biological Oxygen Demand curve using same initial seed concentration as in the simulated stream (5 ml sewage/1).

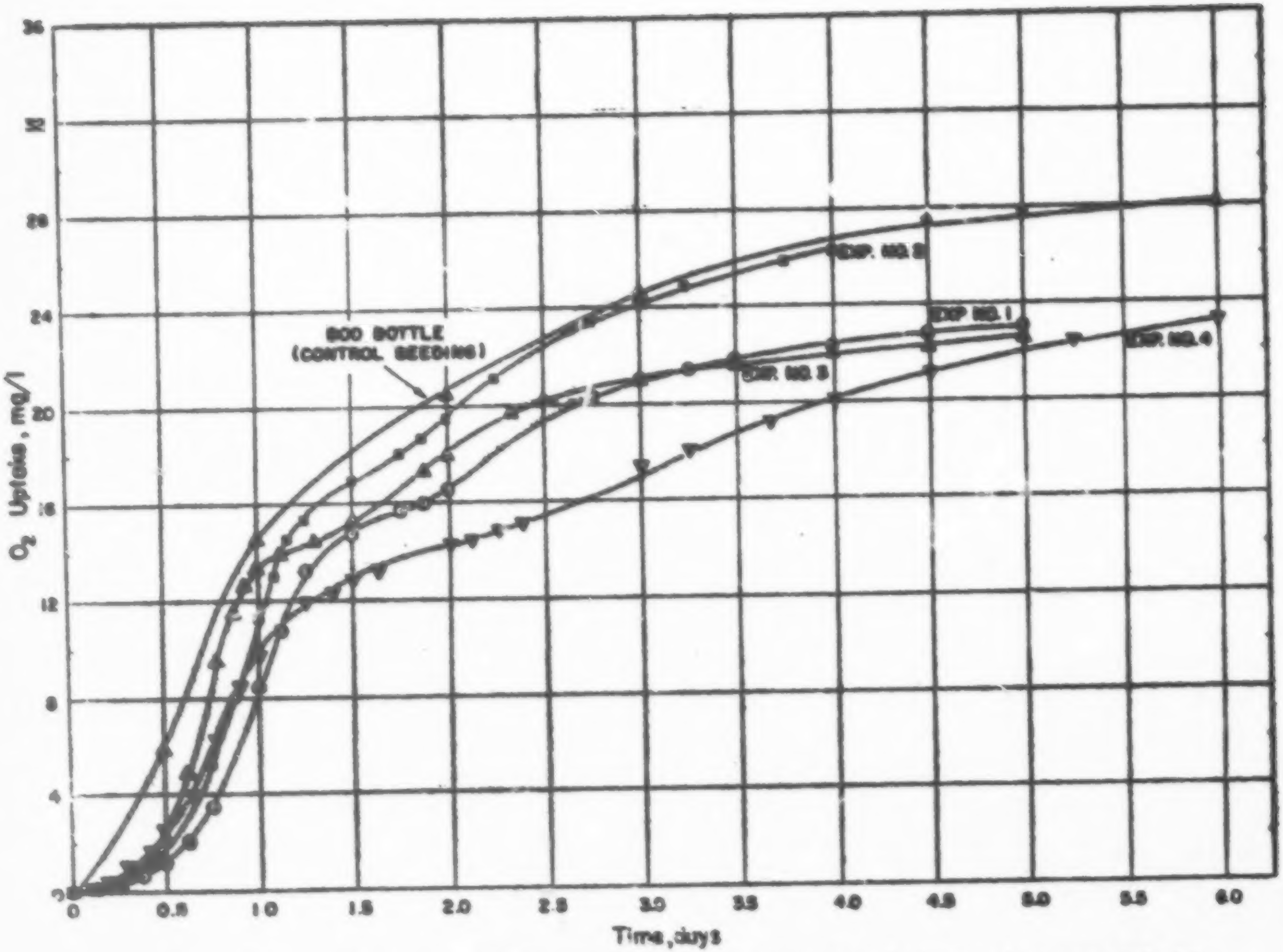

Fig. 10. Comparison of BOD exertion in the simulated stream and in BOD bottles using same seed concentration.

DISCUSSION

In these studies a simulated river has been used to study the course of reaeration and deoxygenation for comparison with the deoxygenation or BOD progression curve generated employing the standard BOD bottle technique. Although there were some general similarities in the oxygen uptake curves obtained from both types of systems, there were distinct differences which appear to be dependent upon either the degree of turbulence in the system or the concentration of initial biological solids, or both. The longer lag in the BOD bottle system may be attributed, in accordance with the results of the present investigation, to differences in initial seed concentration. In general, three distinct phases of oxygen exertion were observed in both types of systems. In the simulated river system it was shown that the first phase of oxygen exertion was associated with removal of initial substrate and concurrent increase in biological solids concentration (see Figure 4). These results are in agreement with results reported from the authors' laboratory and by others, using systems other than those which simulate receiving streams (4). In these studies, the primary difference between the turbulent and quiescent systems during the first stage of oxygen uptake appeared to be the partitioning of substrate between cell synthesis and respiration.

Using the data shown in Figure 4, one can make an oxygen balance for the system at the time of the peak in the biological solids concentration curve. During the period of time preceding this point (thirty-one hours) there was a net increase of 18 mg/l in the solids concentration. Gaudy, Bhatla & Gaudy (11) found that young cells grown on a minimal glucose medium, similar to the one used in the present studies, exhibit an average equivalent COD of 1.52 times the cell weight. Utilizing this value, the amount of equivalent COD channelled into cell synthesis is equal to 27.4 mg/l. During the same period of time the oxygen uptake was 14.6 mg/l of equivalent COD. A mixture of 22 mg/l glucose and 22 mg/l glutamic acid yields a theoretical COD of 44.8 mg/l. Summing the equivalent COD channelled into cell synthesis (27.4 mg/l) and into respiration (14.6 mg/l) the calculated recovery is approximately 93.5 per cent of the original COD of the substrate. These figures also yield a ratio of synthesis to respiration of approximately 1.9:1. In this experiment, oxygen uptake in the BOD bottle system during the first stage was approximately 21.2 mg/l or 47.3 per cent of the theoretical COD, indicating a probable ratio of synthesis to respiration of approximately 1:1.

For the experiment in which the reaeration rate, k_2, was approximately twice the value used in other experiments (see Figure 5), the total first stage oxygen demand was approximately 12 mg/l, or 26.9 per cent of the theoretical COD of the original substrate. Biological solids and substrate concentration were not determined during this experiment, but if one assumes that at the end of the first stage of oxygen uptake the substrate has been removed and the solids concentration curve is at its peak (as shown in Figure 4 and in many other experiments in the authors' laboratory and in others), the remaining 73.1 per cent may be attributed to cell synthesis, yielding a ratio of synthesis to respiration of approximately 2.7:1 for the more turbulent system.

The possible significance of the effect of turbulence in comparing ratio of synthesis to respiration for the quiescent BOD bottle system and the receiving stream cannot yet be fully delineated. It is noted that Gaudy, Bhatla, Follett, and Abu-Niaaj (12) found that the percentage of the theoretical oxygen demand of the substrate exerted during the first stage of oxygen uptake fell within the range of 26 to 51 per cent. It may be seen in the last column of Table I that all values obtained in the simulated river system for the present investigation falls within this range; thus, it cannot be concluded that the lower value was due solely to the effect of turbulence.

In analyzing the results, an attempt was made to determine whether a mathematical model or a kinetic approach could be devised which would reproduce the results observed in the simulated stream system. It was discerned in other portions of these studies that the usual first order kinetic concept was indeed valid for predicting k_2 in the simulated stream. Concerning BOD exertion, it was found that during the first stage of oxygen uptake the usual first order decreasing rate kinetics could be employed to make an adequate engineering prediction according to the following equation:

$$\frac{dL_p}{dt} = -k_1'L_p \qquad (9)$$

TABLE I

Experiment No.	Substrate	1st Stage Exertion % Theoretical COD	2nd Stage Exertion % Ultimate BOD
1	Glucose - Glutamic Acid	39	25
2	Glucose - Glutamic Acid	40	35
3	Glucose - Glutamic Acid	34	32
4	Glucose - Glutamic Acid	27	45
6	Glucose	38	31

Percentages of theoretical COD and ultimate BOD expressed during first and second stages of oxygen uptake in the simulated stream.

In this equation L_p is the amount of the first stage of BOD remaining at any time, t, which reflects the respiration associated with the removal of the original exogenous substrate, and k_1' is the first order decreasing rate constant associated with the first phase of oxygen demand. It is emphasized, however, that in fact this stage does not follow first order decreasing rate kinetics, but is and should be expected to be an autocatalytic curve. All else being equal, the true shape of the curve in the first stage of oxygen uptake will depend to a large extent upon the initial seed or active biological solids concentration.

Following the first stage of oxygen uptake, the curve progresses through the so-called "plateau" period. This "plateau" period may be considered as a stage of negligible oxygen uptake rate in accordance with previous findings (4). This stage is followed by a second rather significant exertion of oxygen demand which, as seen in Figure 4, is associated with a decrease in biological solids concentration. In the present studies it was noted that this phase of oxygen uptake was always accompanied by a distinct clarification in the turbulent system. Also, examination under the microscope showed that an abundance of protozoa were present during this stage of O_2 uptake. These observations are in general agreement with the results of the detailed studies performed by Bhatla and Gaudy pertaining to the role of the protozoa in the exertion of second stage carbonaceous BOD (4). These workers reported that the second stage exertion was approximately thirty per cent of the ultimate BOD exerted. It can be seen from Table I that the values obtained in this investigation are of the same order of magnitude. From their results, Bhatla and Gaudy concluded, in agreement with Busch, that the protozoa could "mask" the plateau. They also concluded from their results that the extent or length of the plateau period was proportional to the relative time lag between the peaks in the bacterial and protozoan populations. The time lag between the peaks in the two types of populations is not unequivocally dependent upon the relative numbers of each in the initial seed, since not all bacteria are substrates for all protozoa. Bhatla and Gaudy were also able to show that, although there are other valid and experimentally demonstrable causes for a plateau in the BOD curve, they are of rather rare occurrence compared to plateaus caused by a time lag in the sequential growth of the bacteria and protozoa (4).

In any event, based upon the previous work and observations made during the course of the present investigation, there appears to be little doubt that the second major oxygen uptake in the simulated stream was due to the use of the bacterial populations as food by the protozoan population. Examination of the second stage of oxygen uptake in the turbulent systems indicated that for engineering purposes, the rate of O_2 utilization could be approximated by zero order kinetics:

$$\frac{dY_S}{dt} = C_S \qquad (10)$$

In this equation Y_s is the amount of oxygen uptake exerted at any time, t, during the second phase, and C_s is the zero order rate constant associated with the second phase of oxygen uptake. It is emphasized again that this curve, like that for the first stage of oxygen uptake, would be expected on a theoretical basis to be an autocatalytic one. However, in the present experimentation it could be approximated for the purposes of making engineering calculations by a zero order equation.

As oxygen uptake progressed beyond the somewhat rapid second stage, there appeared to be a rather slow tailing-off stage, which the authors have chosen to term an endogenous respiration phase. Like the second phase, the third stage or endogenous phase could, for the purpose of attempting to make an engineering prediction, be considered to follow a zero order kinetic expression. Again, it is emphasized that the curve that could be expected on a theoretical basis for endogenous respiration would not be zero order except in the very early stages of endogenous respiration. One would not expect a constant rate but a constantly decreasing rate as more cells begin to die in the system, or otherwise exhaust internal carbon stores. One would expect that the rate of oxygen exertion in this phase would be dependent upon the amount of the bio-mass present, and upon the ability of the bio-mass to use stored and cellular components and those released by lysis. Also, one might expect a correlation between the amount of bio-mass present and the amount of organic matter initially introduced into the stream. For the purposes of making engineering computations, the third stage of oxygen uptake may be approximated by a zero order curve similar to that used to express the second stage of oxygen demand:

$$\frac{dY_E}{dt} = C_E \qquad (11)$$

In this equation Y_E is the amount of third stage oxygen uptake at any time, t, and C_E is the zero order rate constant associated with this demand. It was felt that a rather clear distinction between the second and third stage could and should be made, since the second stage was associated with a rather sharp decline in biological solids concentration in the system whereas the third stage was associated with a very gradual decline in biological solids concentration.

Figure 11 shows the results of an attempt to duplicate the observed dissolved oxygen sag curve for the first experiment (Figure 2) by application of equations (9), (10), and (11). During the first stage of oxygen exertion, the classical sag equation was employed since the data could be approximated by a first order decreasing rate equation for deoxygenation. During the second phase, zero order deoxygenation and first order reaeration were employed as follows:

$$\frac{dD}{dt} = C_S - K_2D \qquad (12)$$

This equation may be integrated to yield the following expression:

$$D_t = \frac{K_2D_o - C_S}{K_2e^{K_2t}} + \frac{C_S}{K_2} \qquad (13)$$

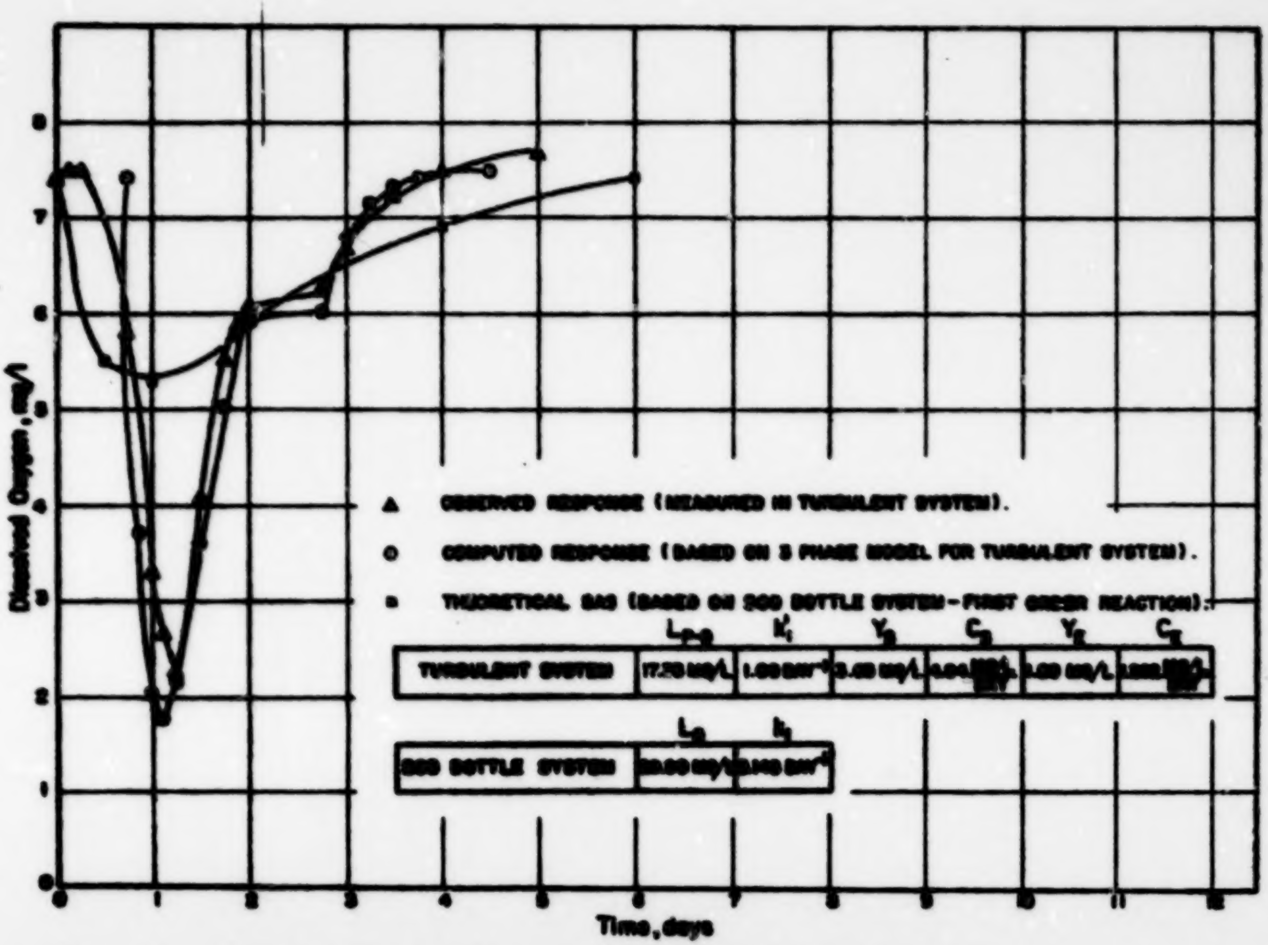

Fig. 11. Comparison of observed and computed sag curves.

In employing this equation, t_0 was adjusted to coincide with the beginning of the second phase, and the initial deficit, D_o, was taken to be the observed deficit at the end of the first stage of O_2 exertion, or the beginning of the second stage exertion, since these two points were assumed to coincide. The third stage of oxygen uptake was approximated by the following differential equation:

$$\frac{dD}{dt} = C_E - K_2 D \tag{14}$$

which can be integrated to yield the following expression:

$$D_t = \frac{K_2 D_o - C_E}{K_2 \, e^{K_2 t}} + \frac{C_E}{K_2} \tag{15}$$

Again, zero time was adjusted to the end of the second stage exertion or beginning of the third stage exertion. The initial dissolved oxygen deficit D_o was taken as the dissolved oxygen deficit at this point. Also, in making calculations using these three equations, an initial lag period of 0.75 day was assumed prior to the first stage of oxygen exertion in order to correct for the observed lag period.

It can be seen from Figure 11 that the value for dissolved oxygen computed in this manner approximates very closely the actual observed DO sag curve. It is also seen that the theoretical sag curve based upon the usual first order decreasing rate for BOD exertion determined from BOD bottle data, was totally inadequate for describing the observed dissolved oxygen sag curve.

It was of interest to determine if O_2 uptake data obtained in the BOD bottle could be used to predict the dissolved oxygen sag in the simulated stream using the same computational procedure as that used for the results shown in Figure 11. The BOD data selected were those shown in Figure 9. It will be recalled that these BOD data were obtained using the same seed concentration as that used in the simulated stream (5 ml/l) and it can be seen from Figure 10 that the use of the same seed concentration did produce an O_2 uptake curve of roughly the same magnitude as was observed in the turbulent systems. However, the BOD curve obtained from the bottle data did not exhibit distinct phases of O_2 uptake. Therefore, in order to use the computational procedure, it was necessary to estimate the magnitude and extent of the phases. The data of Figure 9 are reproduced in Figure 12, which also shows the estimated extent of the assumed three phase exertion. Many previous experiments have shown that the extent of the first phase (when it can be discerned from the O_2 uptake curve) is in the range of 30 to 40 per cent of the theoretical oxygen demand of the substrate (12). A value of 40 per cent was assumed, since it lies within the range of values previously determined and agrees fairly well with the work of others (13). It can be seen from Figure 12 that estimates of second and third stage exertion as zero order reactions yield at best crude approximations for the O_2 uptake curve obtained in the BOD bottle experiment. Figure 13 shows the predicted sag curve based upon the kinetic constants for BOD exertion calculated from the data of Figure 12. All values are given in the table on the figure.

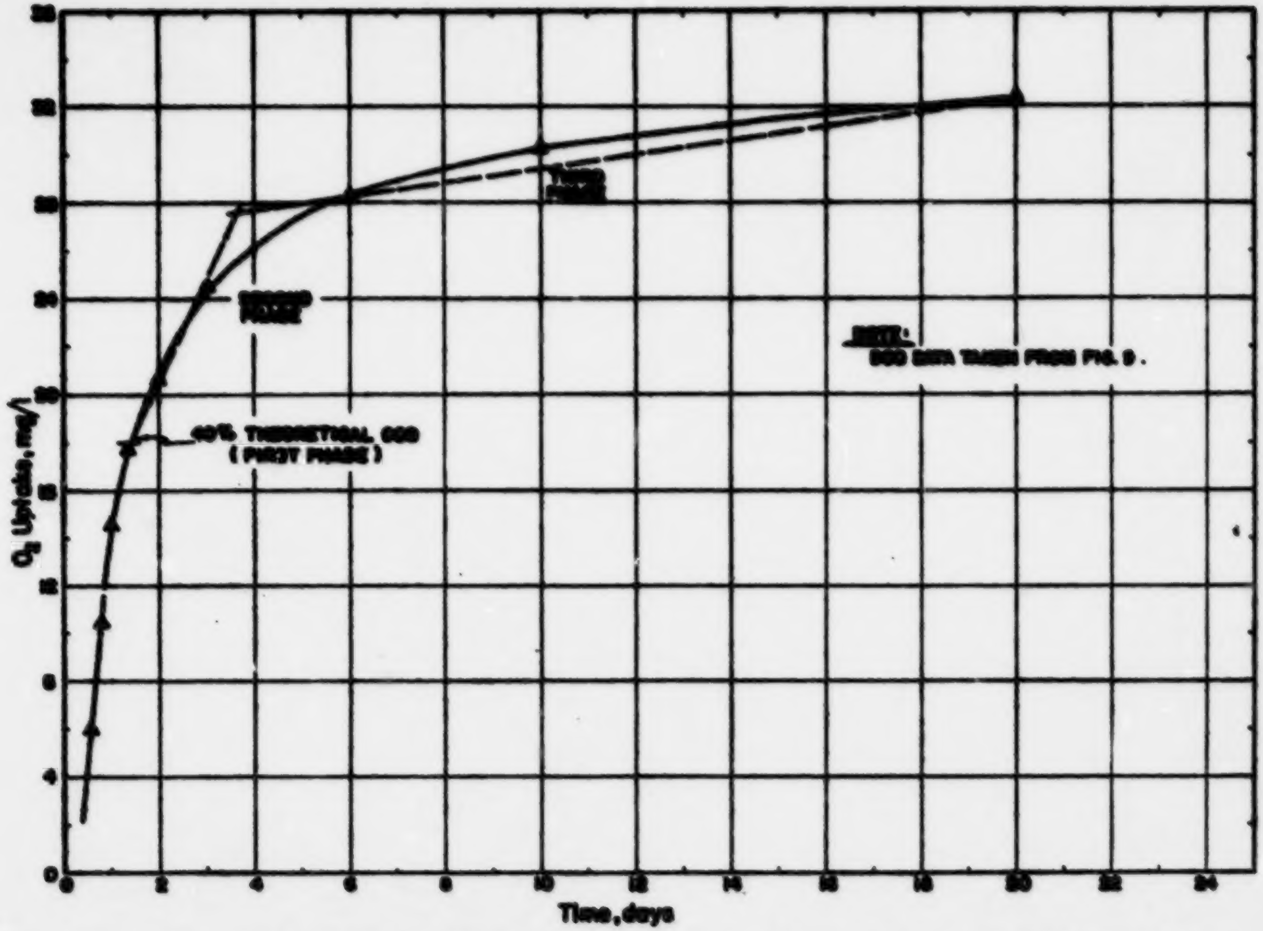

Fig. 12. Estimate of first, second, and third phases of BOD exertion.

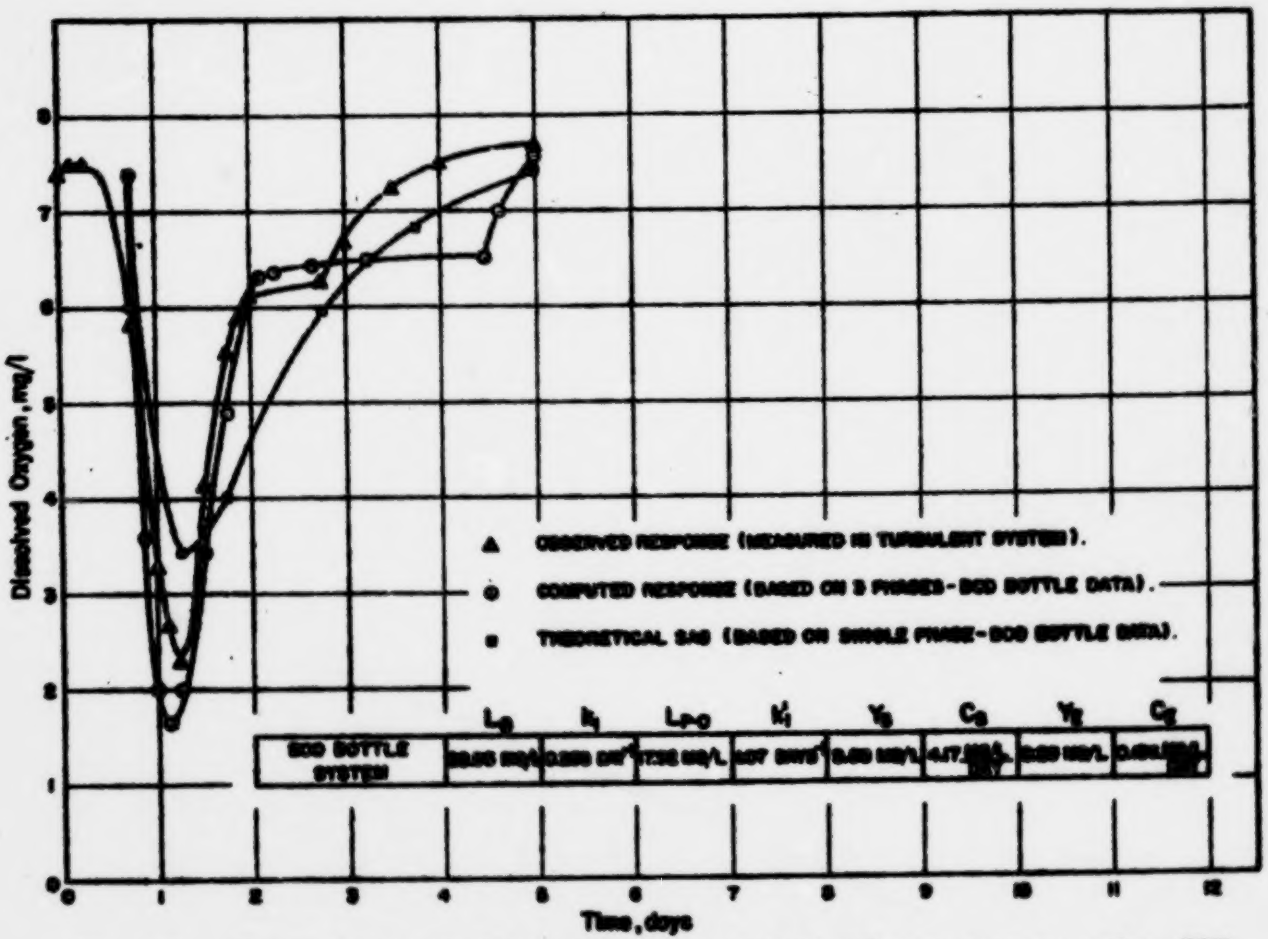

Fig. 13. Comparison of observed and computed DO sag curves using BOD bottle data of Fig. 12.

The three phase computation was made in the same manner as that used for the predicted curve shown in Figure 11. Also shown is the predicted sag curve calculated on the assumption that one first order rate equation determined from the BOD bottle data can be used to predict the sag. The observed oxygen concentration in the simulated stream is also shown (same as Figure 11). It is seen that the curve computed on the basis of three phase exertion approximates fairly closely the point of minimum dissolved oxygen which was observed in the turbulent system. The level of dissolved oxygen concentration during the second stage is also approximated reasonably well using the BOD bottle data; however, the total period of the second stage demand is larger than that for the observed DO curve. Prediction of the time period for the second and third stages is unsatisfactory, largely because the shape of the BOD bottle exertion curve yielded such a gross approximation of zero order kinetics for these stages. Such was not the case for O_2 uptake in the turbulent systems.

Also shown in this figure is the theoretical sag curve calculated on the assumption that one first order rate equation determined from the same BOD bottle data can be used to predict the sag curve. It may be readily determined that the use of the traditional sag equation does not adequately predict the actual sag observed in the turbulent system. Furthermore, it predicts a less severe DO sag than actually occurred in the critical region, i.e., the region of minimum DO.

SUMMARY AND CONCLUSIONS

In summary, it has been shown that phasic exertion of biochemical oxygen demand occurs in turbulent systems under physical conditions similar to those which exist in receiving streams. The dissolved oxygen concentration curve in the simulated receiving stream could be reproduced using three distinct kinetic phases and rate constants derived from O_2 uptake data obtained in the simulated stream. It has also been shown that data obtained in BOD bottles could be used to make a reasonable prediction of the observed minimum or critical DO provided that the seed concentration in the BOD bottle was identical, or nearly so, to the biological solids concentration in the receiving stream at the point of introduction of the synthetic waste, and provided that phasic, rather than one continuous first order, expression was employed to describe BOD exertion. It is significant that no plateau was actually observed in the BOD bottle data which were used in making the prediction of the sag curve in the simulated river system, but a fairly accurate prediction could be made by estimating the extent of the first stage exertion from a knowledge of its extent in systems which did exhibit a plateau marking the end of the first stage O_2 uptake. In conclusion, the results of this experimentation indicate that, although much more work is needed to delineate factors affecting the various rate constants and to define more closely the mechanisms which govern the kinetic expression of oxygen uptake, the approach taken in this work offers some promise of providing adequate prediction of the course of oxygen depletion and recovery in receiving streams.

ACKNOWLEDGEMENT

This work was supported in part by a training grant (5T1-WP-19) and a research grant (WP 00325) from the Water Pollution Control Administration, U. S. Department of the Interior.

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PAPER 21

Goel, K. C., and Gaudy, A. F. Jr., "Regeneration of Oxidative Assimilation Capacity by Intracellular Conversion of Storage Products to Protein." (Submitted for publication in Applied Microbiology, 1968.)

REGENERATION OF OXIDATIVE ASSIMILATION CAPACITY BY INTRACELLULAR CONVERSION OF STORAGE PRODUCTS TO PROTEIN

By

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ABSTRACT

It was known from previous studies that the capacity of a heterogeneous microbial population for oxidative assimilation of glucose could be renewed by periodically subjecting the sludge (or a portion thereof) to endogenous respiration in the presence of an exogenous source of nitrogen. Further study of this process led to a suggested modification of the activated sludge process for nitrogen-deficient wastes. However, it was not known whether renewal of oxidative assimilation capacity was possible for substrates which required the presence of inducible enzyme(s) or for substrates which were not carbohydrates. Therefore, studies were designed using lactose and acetate as carbon sources. Both carbon sources were removed under conditions of oxidative assimilation, and the storage products (or a portion thereof) were converted into protein when the sludge was subjected to a period of endogenous respiration (with respect to carbon source) in the presence of an exogenous supply of ammonium sulfate. The "regenerated" sludge exhibited a renewed capacity for oxidative assimilation, thereby indicating that requisite inducible enzymes (e.g., β-galactosidase in the case of lactose; iso-citritase and malate synthetase in the case of acetate) were not diluted out in the endogenous phase to a degree sufficient to hamper renewed oxidative assimilation capacity. The results also indicated that a non-carbohydrate carbon source could be successfully removed from the medium using this process. However, in the case of acetate, the oxidative assimilation capacity after "regeneration" was not fully restored to the initial level.

INTRODUCTION

In recent studies on the kinetics and mechanisms of waste water purification by the activated sludge process, it was found that, when employing initially high biological solids concentrations, most of the carbon source was incorporated into non-nitrogenous cell constituents during the period of substrate removal. Most of the protein synthesis occurred after the substrate was removed, and the protein was synthesized at the expense of the material (carbohydrate) stored in the cells during the substrate removal period (1)(2). These results indicated that when the feeding population was large, the system behaved much like a nonproliferating system during the period of substrate removal, even though an ample supply of nitrogen was present.

It was reasoned that it might be possible to devise an activated sludge system for certain industrial wastes deficient in nitrogen (e.g., cane sugar refinery and some wood pulping wastes) wherein the carbon source could be removed in the absence of an exogenous source of nitrogen by oxidative assimilation. After separating the accumulated biological sludge from the purified waste, a portion of the sludge could be subjected to endogenous respiration in the presence of an exogenous source of nitrogen, e.g., NH_4^+. During this period a significant portion of the stored material might be converted to protein, thus allowing the sludge to be reused, i.e., recontacted with waste devoid of nitrogen. It was reasoned that such a mode of operation might "regenerate" the oxidative assimilation capacity of the sludge.

Accordingly, experiments using a nitrogen-deficient waste with glucose as carbon source were devised (3). It was found that the sequential synthesis of storage products and proteinaceous cell constituents which occurred at high biological solids concentrations in the presence of nitrogen could, indeed, be physically separated as suggested above, without loss of substrate removal capability. Without a period of endogenous respiration in the presence of an extracellular source of nitrogen, the sludge gradually lost its oxidative assimilation capacity. It was further reasoned that if the system could be run on a continuous basis, it would provide a unique modification of the activated sludge process. Such a process should allow a savings in the cost of supplemental nitrogen (since not all of the sludge would need to be "regenerated") and prevent leakage of nitrogen into the receiving stream (since no nitrogen is added in the purification stage). The latter aspect would be advantageous, since it would help to alleviate the problems attendant to stream enrichment (eutrophication) which are of increasing national concern.

Laboratory pilot plant studies were conducted, using cane sugar refinery wastes, and it was shown that the process was effective under continuous flow conditions (4). Also, additional laboratory studies in continuous flow pilot plants were conducted, using a glucose synthetic waste, in order to delineate the nitrogen requirements of the process and to gain basic information pertaining to the kinetic aspects of this mode of operation (5). It was found that a decided savings in nitrogen could be accomplished.

Although it was possible to conclude that the process showed promise, the previous work left some important questions unanswered. For example, if an early reaction in the metabolism of the waste component depended upon an ample complement of inducible enzyme (such as β galactosidase in lactose degradation), would the endogenous period in the absence of the inducer bring about sufficient dilution or degradation of the enzyme to affect the oxidative assimilation capacity of the sludge when recycled? Also, it was yet to be determined whether the process would work on a non-carbohydrate substrate. Experiments designed to answer these questions are reported in this communication and a following one (6). In the present study, lactose and acetic acid were employed as the carbon sources in batch experiments designed to simulate the nonproliferating feeding phase, the endogenous period in the presence of ammonium ion, and a refeeding phase under nonproliferating conditions.

MATERIALS AND METHODS

The experimental approach and methodology employed were essentially the same as those used in the original batch studies with glucose (3). Activated sludges were developed in batch aerators using an initial seed of settled sewage obtained from the primary clarifier of the municipal treatment plant at Stillwater, Oklahoma. The synthetic waste contained the carbon source (either lactose or acetic acid) and the following inorganic salts: 1.0 M potassium phosphate buffer, pH 7.0, 20 ml/l; $(NH_4)_2SO_4$, 1000 mg/l; $MgSO_4 \cdot 7H_2O$, 200 mg/l; $FeCl_2 \cdot 6H_2O$, 1.0 mg/l; $MnSO_4 \cdot H_2O$, 20 mg/l; $CaCl^2$, 15 mg/l; tap water, 200 ml/l, and distilled water to volume. The batch activated sludge was developed as follows. Daily, one-third of the aeration liquor (500 ml) was wasted and the remainder (1000 ml) was allowed to undergo quiescent settling for one hour; 500 ml of supernatant were wasted and the volume was made up to the original 1500 ml with the synthetic waste. The systems were run at $25^{o} \pm 2^{o}C$ at an air flow rate of 2400 cc/min. After 15 days of such operation, the systems had attained solids balance and were used for experimentation. The cells were harvested, washed twice in phosphate buffer-salts medium, devoid of nitrogen and carbon sources, and subjected to the experiments outlined below.

Feeding Phase: The washed sludge was divided into two equal portions. Carbon source and buffer-salts medium containing ammonium sulfate was added to one portion (growth system) and the ammonium sulfate was withheld from the other ((nonproliferating system). Both liquors were aerated under identical conditions, and samples were withdrawn periodically for determination of biological solids concentration (membrane filter technique, 0.45 μ pore size), protein (biuret) and carbohydrate (anthrone) content of the sludge (7). The removal of the carbon source was assessed by determining the chemical oxygen demand (COD) of the filtrate (8) and in experiments using lactose, substrate removal was also determined by the anthrone test (7).

Endogenous Phase: After completion of the feeding phase (exhaustion of the carbon source), the biological solids from both aeration tubes were harvested and washed in buffer-salts medium devoid of nitrogen source. Both sludges were again divided into two portions; one of each of the subdivided portions was subjected to endogenous respiration in the presence of ammonium sulfate, while the ammonium ion was withheld from the other portion. This procedure resulted in four endogenous systems (with and without nitrogen source for both the growth and the nonproliferating feeding phase reaction liquors). During the endogenous phase, samples were withdrawn for determination of the protein and carbohydrate content of the sludge.

Refeeding Phase: At the end of the endogenous phase, all four sludges were harvested, washed, and resuspended in buffer-salts medium containing carbon source but no nitrogen source and subjected to a refeeding phase during which the substrate removal and sludge accumulation were assessed.

It may be discerned that for the four systems described above, the experimental design for the critical system simulates the nonproliferating feeding phase, endogenous sludge regeneration in the presence of nitrogen source, and recirculation of the protein-enriched sludge which would occur in a continuous flow activated sludge plant operated under the proposed scheme. The other three systems were examined for comparative purposes. For studies employing lactose, preliminary experimentation indicated that the two systems undergoing endogenous respiration in the absence of the nitrogen source did not add significant information, and they were omitted in the final experiments on lactose.

RESULTS AND DISCUSSION

Studies using Lactose

Feeding Phase: Figure 1 shows substrate removal and sludge accumulation during a feeding phase under growth conditions (A) and under nonproliferating conditions (B). Both systems exhibited an excellent waste purification capacity (approximately 90 per cent COD removal). Substrate removal took place at a slightly more rapid rate in the growth system. However, it should also be noted that the nonproliferating system started with slightly lower biological solids and more carbon source. On the basis of calculation of COD removal rate as mg/l COD removed/hour/mg/l of initial biological solids, the systems were for all practical purposes, equally effective. The yield of biological solids was approximately the same (slightly less than 0.6 mg/l cells per mg/l COD removed) under both modes of operation. Also, it is interesting to note that under growth conditions, at the high initial concentration of biological solids employed, the synthesis of carbohydrate preceded protein synthesis. Under nonproliferating conditions, there was no net synthesis of protein but significantly more carbohydrate was synthesized than under growing conditions. These results are in general accord with those of the original experimentation employing glucose (3).

Endogenous Phase: After four hours of aeration in the feeding phase, the cells from both units were harvested, washed, and subjected to endogenous respiration in the presence of ammonium sulfate for six hours. During this period, the

Figure 1 - Removal of substrate and changes in concentrations of biological solids and cellular protein and carbohydrate with lactose as carbon source:
A - Under growth conditions
B - Under nonproliferating conditions.

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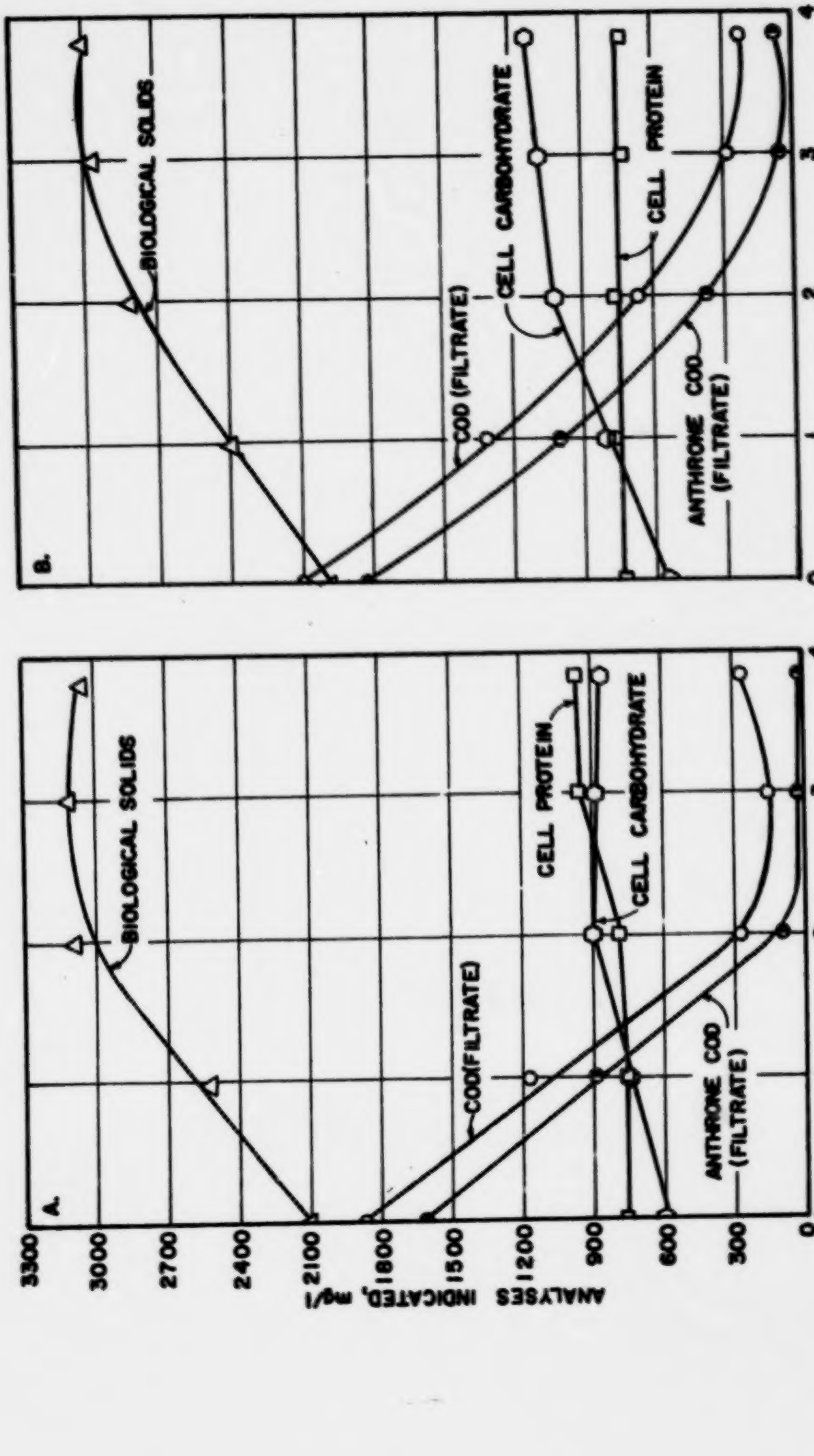

A.
B.
ANALYSES INDICATED, mg/l
3300
3000
2700
2400
2100
1800
1500
1200
900
600
300
0
TIME, HOURS
0
1
2
3
4
BIOLOGICAL SOLIDS
COD(FILTRATE)
ANTHRONE COD (FILTRATE)
CELL PROTEIN
CELL CARBOHYDRATE
TIME, HOURS
0
1
2
3
4
BIOLOGICAL SOLIDS
COD (FILTRATE)
CELL CARBOHYDRATE
CELL PROTEIN
ANTHRONE COD (FILTRATE)

biological solids concentration decreased only slightly, and there was an increase in protein content and a decrease in carbohydrate content in both systems. In the system of major interest, i.e., cells from the nonproliferating feeding phase, the protein content increased from 24 to 34.2 per cent, and the carbohydrate content decreased from 37.5 to 27.8 per cent. These results provided ample evidence that carbohydrate stored in the feeding phase was converted to protein in the endogenous phase in the presence of an extracellular source of nitrogen.

Refeeding Phase: At the end of the endogenous period, cells from both systems were harvested and washed, and a portion of each was subjected to a refeeding of lactose under nonproliferating conditions. The results are shown in Figure 2. It is seen that there was essentially no difference in the substrate removal and sludge synthesizing capabilities of cells which had originally been under growth conditions in the feeding phase (A) and cells which had originally been under nonproliferating conditions (B). Of greater significance is the fact that comparison of systems B in Figures 1 and 2 reveals that the oxidative assimilation capacity of the biomass was not impaired due to the endogenous period of intracellular conversion of carbohydrate to protein.

Studies using Acetic Acid

Feeding Phase: It is seen in Figure 3 that acetic acid was removed quite rapidly and at the same rate with approximately the same accumulation of biological solids under growing and nonproliferating conditions. In the growth

Figure 2 - Biological solids production and substrate removal under nonproliferating conditions using lactose as carbon source:

A - Biological solids originally subjected to growth conditions and subsequently subjected to endogenous respiration in the presence of NH_4^+

B - Biological solids originally subjected to nonproliferating conditions and subsequently subjected to endogenous respiration in the presence of NH_4^+ .

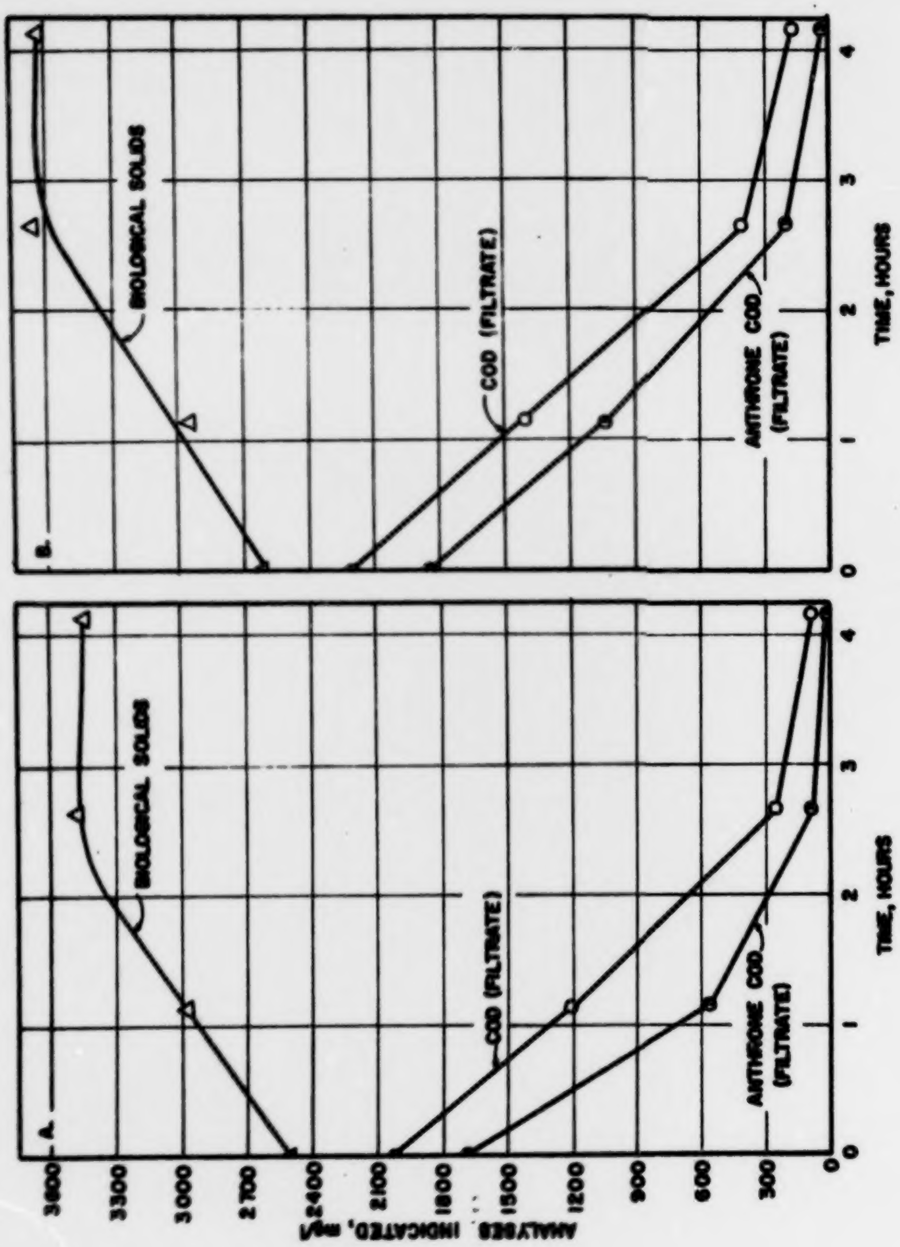
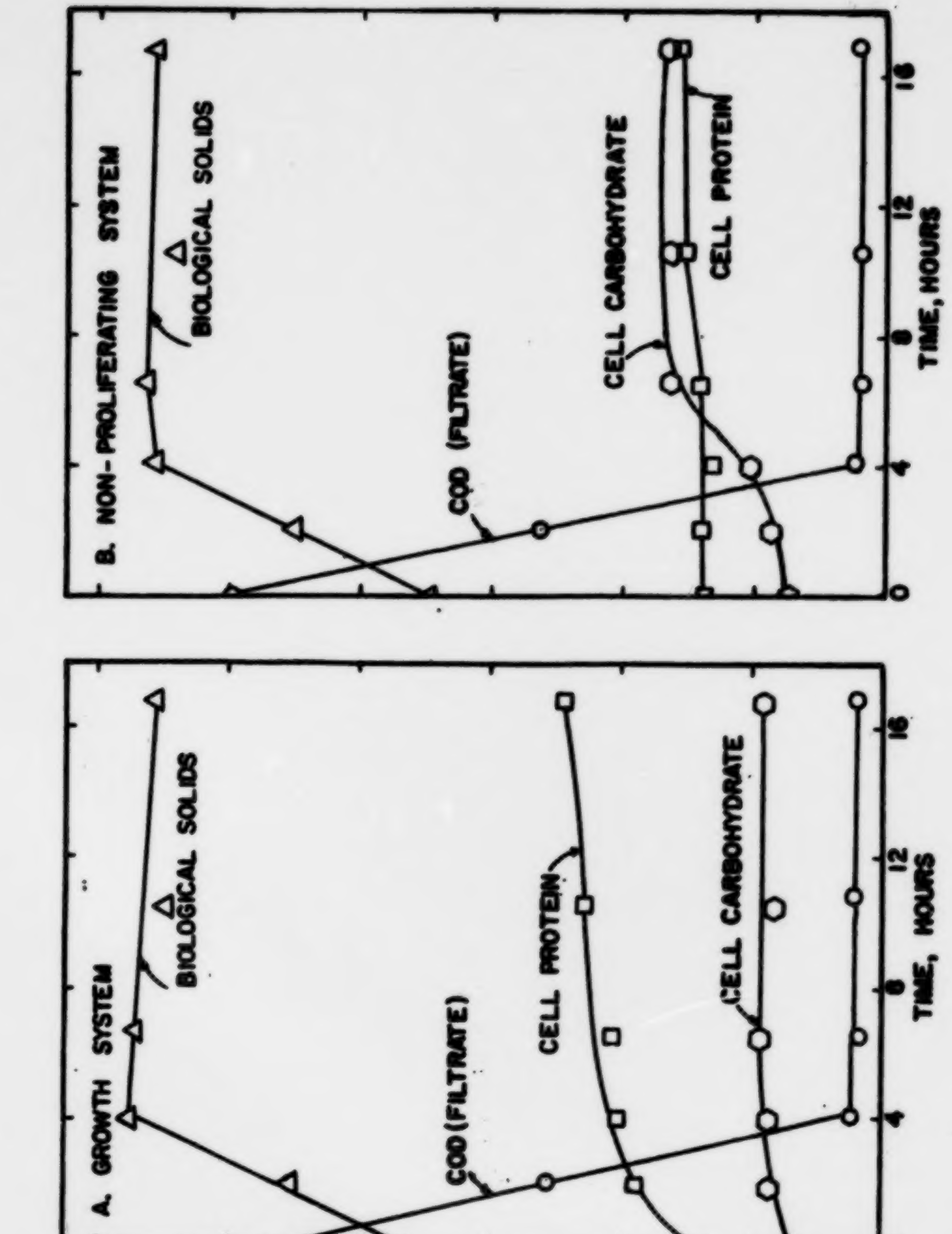

A.
B.
BIOLOGICAL SOLIDS
COD (FILTRATE)
ANTHRONE COD
(FILTRATE)
ANALYSES INDICATED, mg/l
0
300
600
900
1200
1500
1800
2100
2400
2700
3000
3300
3600
TIME, HOURS
0
1
2
3
4

Figure 3 - Removal of substrate and changes in concentrations of biological solids and cellular protein and carbohydrate with acetate as carbon source:
A - Under growth conditions
B - Under nonproliferating conditions.


A. GROWTH SYSTEM
B. NON-PROLIFERATING SYSTEM
BIOLOGICAL SOLIDS
COD (FILTRATE)
CELL PROTEIN
CELL CARBOHYDRATE
ANALYSES INDICATED, mg/l
0
400
800
1200
1600
2000
2400
TIME, HOURS
0
4
8
12
16

system, cellular carbohydrate concentration increased from 275 to 360 mg/l (as a percentage of the dry weight of biological solids it decreased from 18.5 per cent to 15 per cent and finally increased again to 16.5 per cent), whereas in the nonproliferating systems the concentration of cellular carbohydrate increased to 630 mg/l (final carbohydrate content was 29.6 per cent). In the nonproliferating system, it is interesting to note that during the period of rapid removal of the carbon source and sludge accumulation (first four hours) the cell carbohydrate did not rise rapidly. It would appear that for these acetic acid acclimated cells, the immediate storage product was not carbohydrate. Most of the carbohydrate was synthesized after the carbon source had been removed and the biological solids concentration had leveled off, indicating that the carbohydrate was synthesized as a result of intracellular conversion of an initial storage product. The lipid content of the cells was not assessed, and it can therefore be only surmised that for the oxidative assimilation of acetate, lipids comprised the immediate carbon store under the conditions of the experiment.

Endogenous Phase: At the conclusion of the feeding phase, both sludges were harvested, washed, and subdivided into the four endogenous systems described earlier. In the most critical system, i.e., sludge harvested from the nonproliferating feeding phase and subsequently subjected to endogenous respiration in the presence of ammonium sulfate, the carbohydrate content decreased from 29.6 per cent to 21 per cent; however, the rise in protein content was rather small, from 27.9 per cent to 30 per cent.

Refeeding Phase: All four endogenous systems were harvested and washed, and portions of each sludge were subjected to the nonproliferating refeeding phase. Only a part of each sludge was used in order to prolong the substrate removal phase and allow more accurate characterization of the kinetics of removal. Since the systems were to be judged on a comparative basis, the initial solids level was not critical for assessment of the results. The substrate removal characteristics of each system are shown in Figure 4. Because of the relatively low initial biological solids concentration used in this phase, it is not possible to compare directly the rates of COD removal in Figure 4 with those of Figure 3. However, from Figure 3 the rate of COD removal for the growth system expressed in terms of mg/l COD removed/hour/mg/l initial biological solids was found to be 0.3, whereas it was 0.34 for for the nonproliferating system; i.e., the rates were approximately the same. If the COD removal rates for the four systems shown in Figure 4 are taken as being approximately linear, comparable calculations for systems A, B, C, and D yield rates of 0.23, 0.21, 0.18, and 0.13 mg/l COD removed, hr/mg/l initial biological solids.

It is seen that system D, which received no nitrogen source in the feeding, endogenous, or refeeding phases, exhibited a sharply retarded oxidative assimilation capacity in the refeeding phase. System C, the most critical one since its

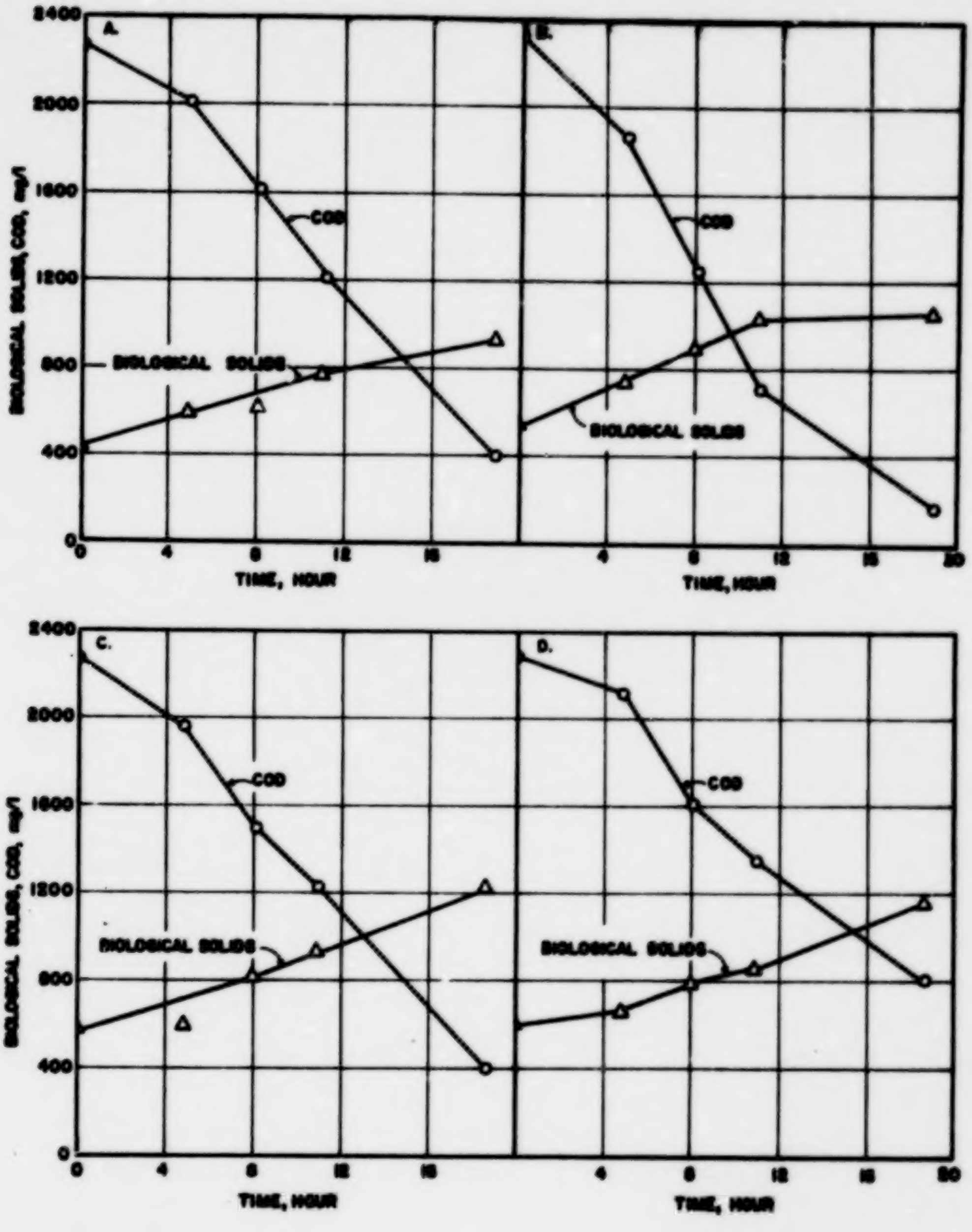
Figure 4 - Biological solids production and substrate removal under nonproliferating conditions using acetic acid as carbon source:

A - Biological solids originally subjected to growth conditions and subsequently subjected to endogenous respiration in the presence of NH_4^+

B - Biological solids originally subjected to growth conditions and subsequently subjected to endogenous respiration in the absence of NH_4^+

C - Biological solids originally subjected to nonproliferating conditions and subsequently subjected to endogenous respiration in the presence of NH_4^+

C - Biological solids originally subjected to nonproliferating conditions and subsequently subjected to endogenous respiration in the absence of NH_4^+.


A.
B.
C.
D.
BIOLOGICAL SOLIDS, COD, mg/l
2400
2000
1600
1200
800
400
0
COD
BIOLOGICAL SOLIDS
0
4
8
12
16
20
TIME, HOUR

past history simulated the proposed modification of the activated sludge process, exhibited a substrate removal capacity in the refeeding phase which was 60 per cent of the value attained in the original feeding phase (i.e., .18/.3). Indeed, the specific substrate removal rates for all systems shown in Figure 4 were significantly lower than the values attained before the endogenous period (Figure 3).

SUMMARY AND CONCLUSIONS

The results using lactose and acetic acid as substrates were, in general, similar to those observed in previous experiments using glucose. It was possible to remove the carbon source in a reasonable time period under nonproliferating conditions. Subjecting the cells to endogenous respiration in the presence of an extracellular source of nitrogen permitted conversion of intracellular storage products to protein and, upon recontacting the cells with substrate under nonproliferating conditions, the cells retained ability to perform oxidative assimilation of the carbon source. In the case of lactose, it was seen that acclimation to the substrate was not impaired by the period of endogenous respiration. With acetic acid, the oxidative assimilation capacity of the cells was decreased somewhat, apparently due to the endogenous period in the absence of acetate; however, the results left little doubt that a refeeding phase could be successfully accomplished. The results on acetate (compare Figures 3 and 4) also show that if wastewaters are to be treated in accordance with the method suggested by the experimental results, the concentration of biological solids employed should be above the 400-500 mg/l level used in the refeeding phase shown in Figure 4. It is emphasized that the activated sludge aeration tank under the mode of operation herein studied is in no sense a culturing or growth reactor, as it is in a normal activated sludge process. Growth as indicated by a net increase in the feeding population does not occur because there is no readily-available exogenous nitrogen source. The substrate removal mechanism is the oxidative assimilation of the carbon source; the rate at which it can proceed is dependent principally on the number of cells recycled to the feeding reactor from the endogenous phase reactor. Thus, one would expect better results at high rather than low biological solids concentrations.

Although continuous flow studies in completely mixed reactors using both glucose and a cane sugar refinery waste have indicated that the process can be carried out on a continuous basis, the results of batch experiments herein reported for acetate indicate that some difficulty might be encountered using waste of a noncarbohydrate nature. It was felt that further continuous flow studies using acetate would be valuable in ascertaining the possible broader potential applicability of the process. The results of such experimentation are reported in a following article.

ACKNOWLEDGEMENT

This work was supported by a Research Grant WP 00325 from the Water Pollution Control Federation, U. S. Department of the Interior.

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PAPER 22

Gaudy, A. F. Jr., Goel, K. C., and Gaudy, Elizabeth T., "Studies on Continuous Oxidative Assimilation of Acetic Acid and Endogenous Protein Synthesis Applicable to Treatment of Nitrogen-deficient Wastewaters." (Submitted for publication in Applied Microbiology, 1968.)

STUDIES ON CONTINUOUS OXIDATIVE ASSIMILATION OF ACETIC ACID AND ENDOGENOUS PROTEIN SYNTHESIS APPLICABLE TO TREATMENT OF NITROGEN-DEFICIENT WASTE WATERS

By

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ABSTRACT

A previous study indicated that the oxidative assimilation capacity of a heterogeneous microbial population for acetic acid did not return to the initial value after a period of endogenous respiration in the presence of ammonium sulfate. This finding provided cause for restraint in recommending the possible use of a continuous oxidative assimilation process for the treatment of nitrogen-deficient waste waters of non-carbohydrate nature. In order to put the process to a severe test, a laboratory scale pilot plant study was made. Acetate (1000 mg/l) was fed continuously to a completely mixed, aerated reactor vessel, from which the mixed liquor was channelled to a settling basin. The settling basin supernatant was continuously discharged, and a portion of the settled biological sludge was subjected to endogenous respiration in the presence of ammonium sulfate and recycled to the aeration vessel. Experiments were conducted at three levels of ammonia-nitrogen in the endogenous phase. It was found that approximately 90 per cent removal of the carbon source (expressed as chemical oxygen demand, COD) was attained using a low level of supplemental nitrogen (COD/N = 70/1) and a fairly low reactor detention time (four hours). Based upon these and previous results, it has been concluded that the process shows promise for application to the treatment of a wide variety of nitrogen-deficient industrial wastes.

INTRODUCTION

A summary of studies which led to a proposed modification of the activated sludge process for treatment of nitrogen-deficient industrial wastes was included in a previous report (1). Under the proposed mode of operation the nitrogen supplement is not added to the wastewater, as is the usual case, but the waste is contacted with an acclimated biomass (activated sludge) and the carbon source is removed by oxidative assimilation. The biological solids are then settled and the effluent passed to the receiving stream without having come into contact with a source of nitrogen. A portion of the settled sludge is then subjected to endogenous respiration in the presence of an extracellular source of nitrogen (NH_4^+) and the cellular carbon store (or a portion thereof) is converted to protein. The "protein enriched" biomass is then recycled for another contact with the nitrogen-deficient waste. A flow diagram for continuous operation of the process is shown in Figure 1.

Previous studies in batch operation have demonstrated the mechanistic feasibility of the process using synthetic wastes containing glucose (2), lactose (1), or acetic acid (1), and a cane sugar refinery waste (3). Bench scale studies in continuous flow completely mixed reactors have indicated the engineering feasibility of the process using both glucose (4) and a cane sugar refinery waste (3).

However, in the batch studies using acetic acid as the carbon source in the synthetic waste, it was noted that the interconversion of stored carbon to protein in the endogenous phase was less efficient than in studies with other carbon sources, and that the biomass when recontacted with acetic acid under nonproliferating conditions exhibited a decreased oxidative assimilation capacity (1). These results raised a

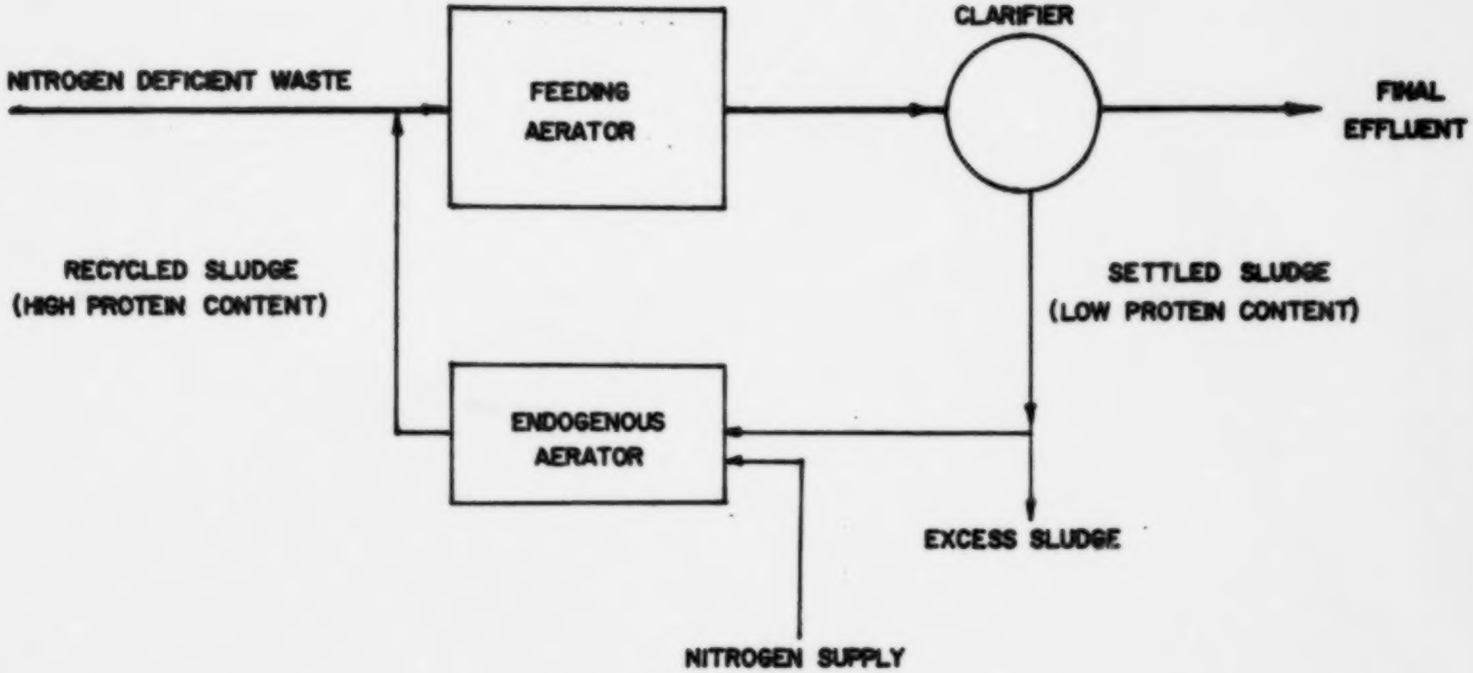

Figure 1 - Flow diagram of process modification for treatment of nitrogen-deficient wastes by activated sludge.

question as to the general applicability of the process to treatment of nitrogen-deficient wastes of a non-carbohydrate character. It was reasoned that continuous flow studies using acetate would provide a fairly rigorous test of the process. If continuous oxidative assimilation of acetic acid could be effected using a reasonable aeration period, broader applicability of the process could be recommended. Accordingly, the research herein reported was undertaken.

MATERIALS AND METHODS

The experimental pilot plant employed in these studies is shown in Figure 2. The volume of aeration liquor in the feeding phase reactor was 2400 ml; a synthetic waste (1000 mg/l acetate as carbon source) was fed at a rate of 400 ml/hr. The aeration liquor flowed to the settling tank from which the supernatant was continuously discharged. At 12-hour intervals sludge was harvested from the bottom of the settling tank and a portion of it was diluted with the supernatant to a predetermined optical density (measured at 540 mμ). This portion of sludge was then channelled to the endogenous aerator for intracellular conversion of stored products to protein. Ammonium sulfate was added, and the sludge was aerated for twelve hours. The sludge was then passed to the sludge holding tank and pumped to the feeding phase aerator at a rate of 200 ml/hr. While sludge was being pumped from the holding tank, another batch of sludge was subjected to regeneration in the endogenous aerator. The feeding phase aerator was operated at a dilution rate of 1/4 hr^{-1}, i.e.,

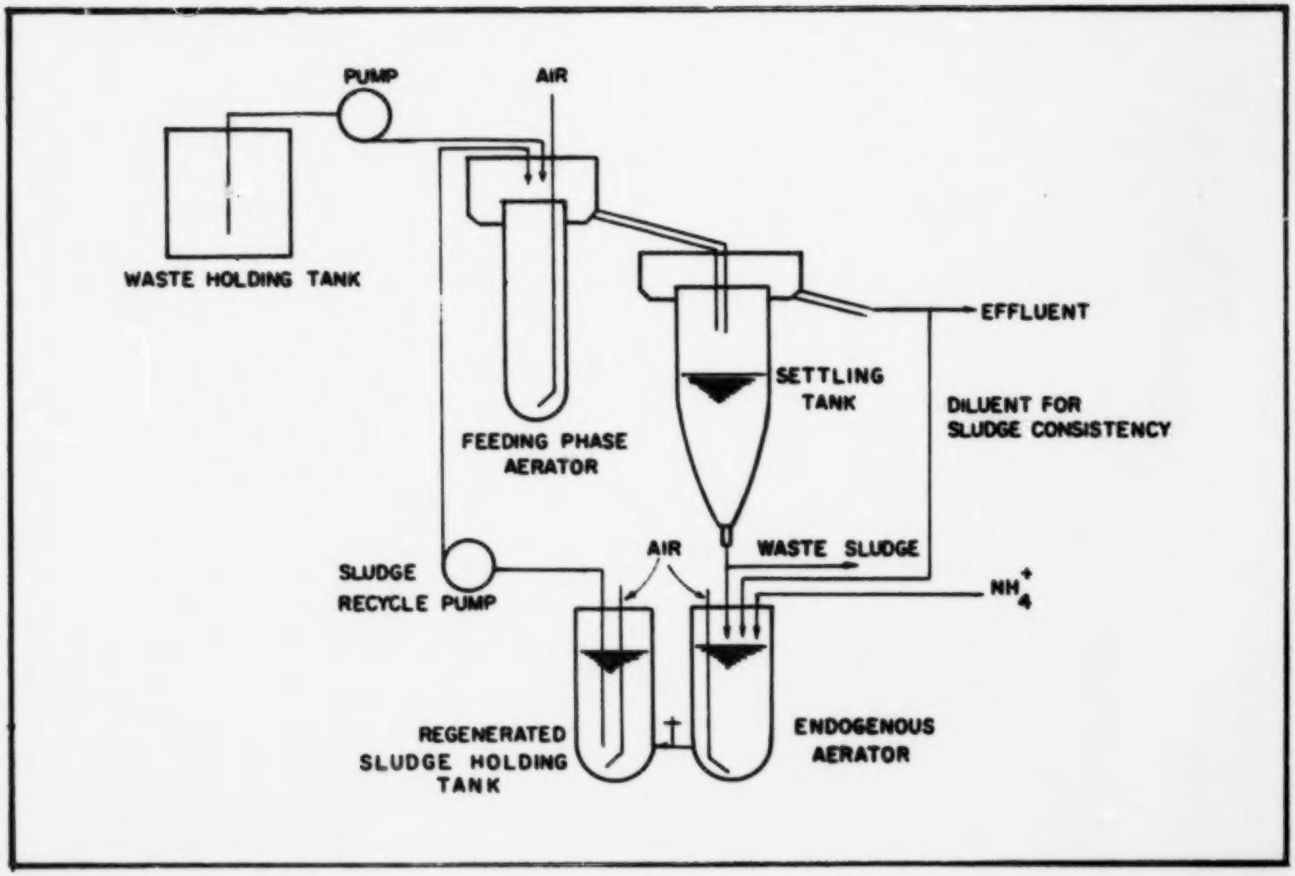

Figure 2 - Laboratory scale pilot plant.

with an inflow of 400 ml/hr synthetic waste + 200 ml/hr recycle solids to a 2400 ml aeration tank volume.

A heterogeneous microbial population was developed using a seed obtained from the primary clarifier effluent of the municipal wastewater treatment plant at Stillwater, Oklahoma. The synthetic waste contained the following constituents: acetic acid, 1000 mg/l; 1.0 M potassium phosphate buffer, pH 7.0, 10 ml/l; $MgSO_4 \cdot 7H_2O$, 100 mg/l; $FeCl_2 \cdot 6H_2O$, 0.5 mg/l; $MnSO_4 \cdot H_2O$, 10 mg/l; $CaCl_2$, 7.5 mg/l; tap water, 100 ml/l, and distilled water to volume.

In conventional activated sludge processes, treatment of nitrogen-deficient wastes is accomplished by adding the supplemental nitrogen source to the inflowing waste at the desired ratio of chemical oxygen demand (COD) to nitrogen, whereas in the process under study, this supplement is not added to the wastewater. In order to compare the nitrogen requirements of these processes, ammonium sulfate was added to the endogenous aerator, at any desired COD/N ratio, on the basis of the total COD supplied to the feeding phase aerator each 12-hour period. The calculated COD of 1000 mg/l of acetic acid is 1060 mg/l (i.e., 1000 mg/l x 64/60); this value agrees with values obtained by the COD test (5). The COD in the incoming feed during a 12-hour period was 5088 mg/l COD (400 ml/hr x 12 hr x 1060 mg/l COD). For operation at a COD/N ratio of 10/1, 508.8 mg ammonia-N would be supplied to the endogenous aerator for each 12-hour period. Since this amount of nitrogen was added to a volume of 2500 ml of sludge, the concentration of exogenous ammonia nitrogen at the start of the endogenous period was 203.5 mg/l. Similarly, for operation at the other COD/N ratios investigated, 30/1, 50/1, and 70/1, the concentrations of exogenous nitrogen source at the beginning of the endogenous period were 67.8, 40.7, and 29.1 mg/l, respectively.

The pilot plants were run at each COD/N ratio for an extended period of time. Periodically, samples of the settling tank effluent were analyzed for COD concentration (5). The aerator mixed liquor effluent was filtered (membrane filter, pore size 0.45μ) and the COD of the filtrate was determined in order to compare the biochemical and total efficiency of the system (i.e., removal of carbon source only, as compared with carbon source removal plus the flocculating and settling characteristics of the cells).

Biological solids concentrations in the feeding aerator mixed liquor, as well as in the cell recycle, were assessed using the membrane filter technique (5). The concentrations of ammonia nitrogen carried over from the endogenous aerator and in the final effluent were determined using the method of Niss, as described by Ecker and Lockhart (6). The protein (biuret) and carbohydrate (anthrone) concentrations of the cells were also determined (7).

RESULTS AND DISCUSSION

The average values of analyses carried out during operation at the three COD/N ratios employed are given in Table I. The average values were obtained from results of extended runs,

TABLE I

AVERAGE VALUES FOR ANALYSES OF EFFLUENT FROM THE FEEDING AND ENDOGENOUS AERATORS DURING CONTINUOUS OPERATION AT VARIOUS COD/N RATIOS

(D - 0.25 hr^{-1}; Carbon Source - 1000 mg/l Acetic Acid - 1060 mg/l COD in the Feed)

COD/N	Effluent from Feeding Aerator											Effluent from Endogenous Aerator				
	Filtrate COD		Supernatant COD (Unfiltered)		NH_3-N in Final Effluent	Sludge	Sludge Yield	Sludge Protein	Sludge Carbohydrate	Sludge Wasted	Carry-over COD	NH_3-N in Carry-over	Sludge	Sludge Protein	Sludge Carbohydrate
	mg/l	% Removal	mg/l	% Removal	mg/l	mg/l	%	%	%	%	mg/l	mg/l	mg/l	%	%
30:1	56	95	95	91	4	822	40	33	20	33	65	55	1659	36	20
50:1	67	94	108	90	1	758	48	30	25	41	76	24	1317	37	27
70:1	98	90	140	87	-	706	48	25	28	43	110	-	1205	36	28

at each COD/N ratio, of the type shown in Figure 3, which shows the operational characteristics of the process when the COD/N ratio was 70/1. In the interest of brevity, detailed operational data for the other two COD/N ratios are not shown. The 70/1 ratio was selected for graphical presentation because it represented the most critical test of the process. Figure 4 shows results typical of the course of intracellular protein production in the endogenous aerator during one of the 12-hour endogenous periods.

In Figure 3, the biological solids in the feeding aerator due to recycle is shown (i.e., recycle sludge concentration x 200/600) rather than the actual solids concentration in the recycle flow. The data were plotted in this way in order to show the net sludge synthesis (hatched area) due to continuous oxidative assimilation. It is seen that continuous oxidative assimilation of acetic acid was effected with a consistently high degree of COD removal. Figure 4 shows that protein synthesis proceeded under endogenous conditions in the presence of an exogenous source of nitrogen. It is also seen that carbohydrate did not provide the primary source of endogenous carbon for protein synthesis.

The results summarized in Table I reveal that good substrate (COD) removal was obtained at all COD/N ratios employed. The efficiency of the system did decrease somewhat (from 95 to 90 per cent removal for filtrate values and from 91 to 87 per cent for supernatant values as the COD/N ratio was increased. The difference between the effluent

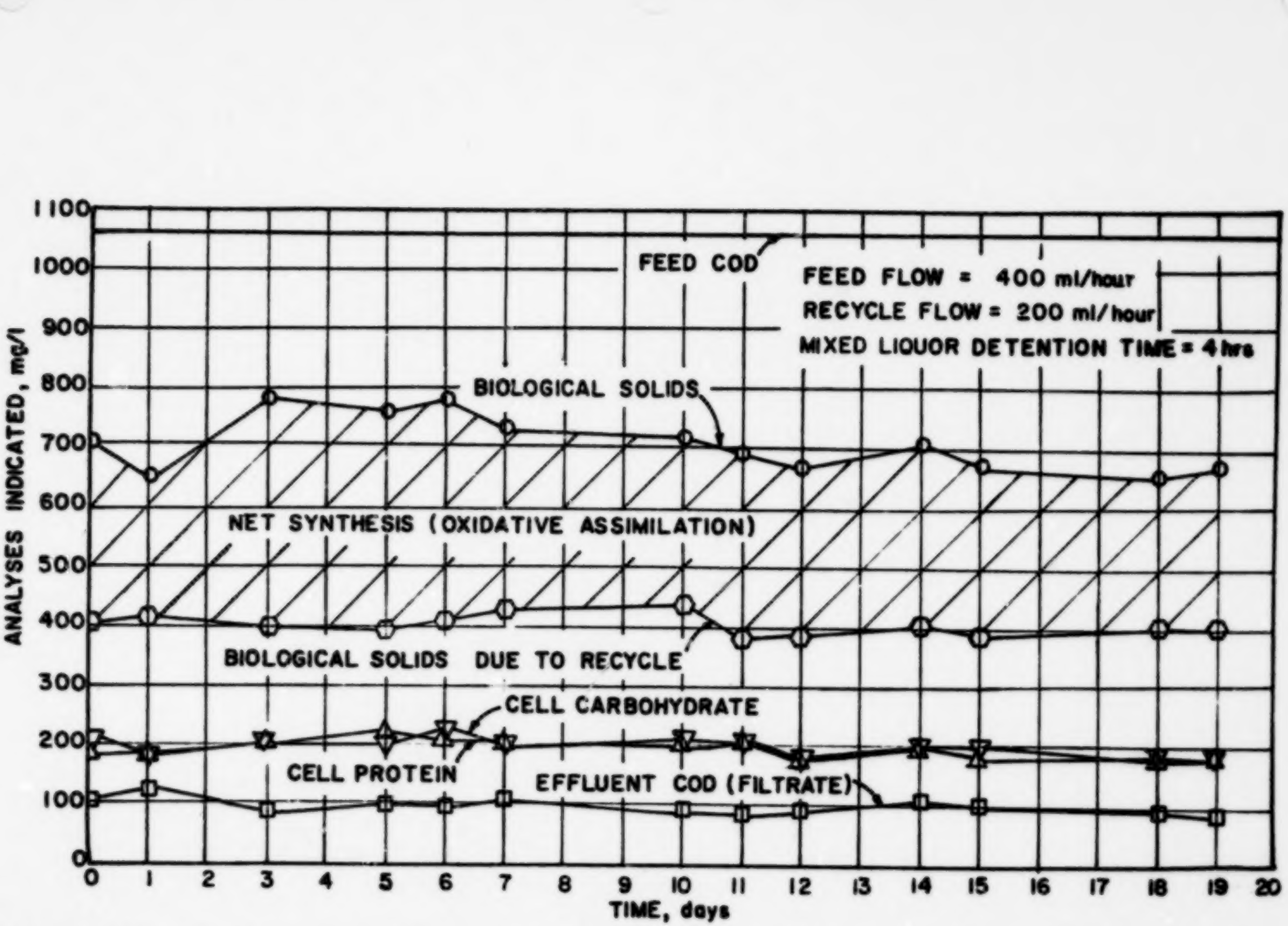

Figure 3 - Continuous oxidative assimilation of acetic acid in the feeding aerator using biological solids previously subjected to endogenous respiration in the presence of NH_3-N (COD/N - 70/1).

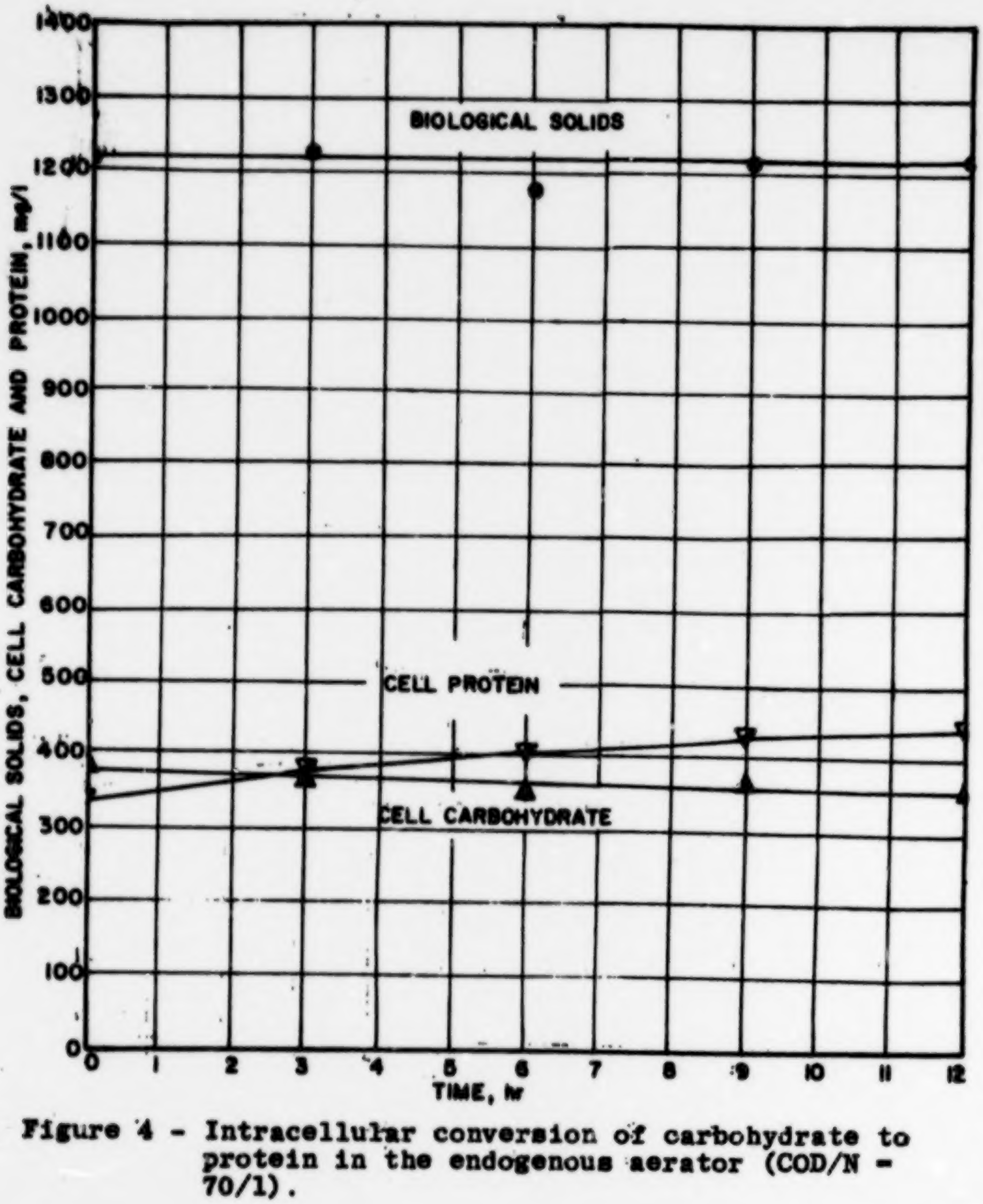

Figure 4 - Intracellular conversion of carbohydrate to protein in the endogenous aerator (COD/N - 70/1).

values for filtrate and supernatant are attributable to cells which did not flocculate and settle in the clarifier. The fairly close agreement between effluent quality values measured by either parameter attests to the rather good flocculating and settling characteristics exhibited by the sludge. It has been determined experimentally that, on the average, the COD of 1 mg of cells is approximately 1.37 mg (8). At the COD/N ratio of 70/1, the COD in the effluent due to unsettled cells was 42 mg/l (i.e., 140 - 98) and the biological solids concentration in the supernatant calculated from the COD equivalent is 31 mg/l (i.e., 42/1.37). The overall flocculation and settling efficiency was therefore approximately 96 per cent, i.e., (706 - 31)/706. In general, the sludge developed on acetate under conditions of oxidative assimilation exhibited good flocculation and settling characteristics throughout the investigation.

Comparison of ammonia-nitrogen concentration in the effluent and that carried over in the recycle sludge indicates that only at the COD/N ratio of 70/1 can it be said that the process was operated under strict oxidative assimilation (or nonproliferating) conditions in the feeding aerator. At the 50/1 level, the concentration of ammonia-nitrogen in the feeding aerator due to recycle was rather small (24 x 200/600 = 8 mg/l) and only 1 mg/l appeared in the effluent.

The cell yield was calculated as the ratio of the hourly rate of sludge production (outflow rate minus inflow rate) to the hourly rate of COD consumption (inflow rate in feed and recycle minus outflow rate). The yield ranged from 40 to 48 per cent, and the COD/N ratio did not exert the rather severe effects on yield observed in studies using glucose as carbon source wherein the yield rose sharply for the low COD/N ratios (4). The percentage of sludge wasted was approximated by calculating the ratio of the average weight of sludge (mg) recycled to the feeding aerator to the average weight of sludge produced for each 12-hour period. For example, at the COD/N ratio of 70/1, the sludge produced in a 12-hour period was 0.6 l/hr x 706 mg/l x 12 hr = 5075 mg, and the weight of sludge recycled was 0.2 l/hr x 1205 mg/l x 12 hr = 2890 mg. The difference (i.e., 2185 mg) represents the weight of sludge which would be subject to further handling and treatment, e.g., digestion and drying, and the comparative parameter "sludge wasted" given in the table is the ratio of this weight to the sludge output (i.e., 2185/5075 x 100 = 43 per cent).

The protein content of the cells in the recycle (effluent from the endogenous aerator) remained fairly constant for all COD/N ratios employed (i.e., 36 to 37 per cent). However, the largest percent increase in protein during the endogenous period took place at the 70/1 level of operation and decreased as more ammonia nitrogen was added to the endogenous aerator. This effect occurred because at the two lower COD/N ratios some of the protein production took place in the feeding aerator due to the carryover of nitrogen from the endogenous aerator. As would be expected (2)(9)(10), the carbohydrate content of the biomass in the feeding phase aerator varied inversely with the nitrogen level in the system, i.e., it increased as the nitrogen level, due to carryover, decreased. Without a source of nitrogen it can be expected that a greater portion of the carbon source would be channelled into non-nitrogenous cell components. It is interesting to note that cellular carbohydrate content did not change to any great extent during the endogenous period (compare sludge carbohydrate in the effluents from the feeding aerator and endogenous aerator). In similar studies using glucose as carbon source, there was a considerable decrease in cell carbohydrate due to its conversion to protein in the endogenous aerator (4). For acetate, however, it would appear that carbohydrate synthesis during oxidative assimilation in the feeding aerator did not provide the primary endogenous carbon store from which protein was synthesized in the endogenous aerator. This finding is in general agreement with the results of the batch studies using acetate (1). Analyses for lipid content were not performed; however, it seems likely that such constituents formed the primary carbon store for protein synthesis in the presence of ammonia-nitrogen in the endogenous aerator. Constituents such as poly-β-hydroxybutyrate could function in such a capacity (11)(12)(13).

CONCLUSIONS

The present study using acetic acid as carbon source was the most critical test to which the proposed modification of the activated sludge process has been subjected. It has been shown that the success of the process does not depend upon use of carbohydrate as the endogenous store during the endogenous phase in the presence of an exogenous source of nitrogen. The carbon source was oxidatively assimilated in the absence of nitrogen, and a sufficient portion was converted to protein under endogenous conditions to allow continuous oxidative assimilation. The process was operated with good substrate removal efficiency (measured as COD on filtrate or supernatant) at a fairly high COD/N ratio (70/1) and a relatively low detention time in the feeding aerator (4 hours). While there is much further work which can be accomplished concerning this process--for example, its efficiency using various concentrations of recycle solids, detention time, etc., and further laboratory studies on whole waste similar to those on sugar refinery wastes (3), it appears from the present study that the process may provide a useful modification permitting a savings in the cost of supplemental nitrogen and curtailment of nitrogen leakage in treatment plant effluents for a wide variety of nitrogen-deficient industrial wastes.

ACKNOWLEDGEMENT

This work was supported by a Research Grant WP 00325 from the Water Pollution Control Administration, U. S. Department of the Interior.

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PAPER 23

Gaudy, A. F. Jr., Goel, K. C., and Gaudy, Elizabeth T., "Application of Continuous Oxidative Assimilation and Endogenous Protein Synthesis to the Treatment of Carbohydrate Wastes Deficient in Nitrogen." (Submitted for publication in Biotechnology and Bioengineering, 1968.)

APPLICATION OF CONTINUOUS OXIDATIVE ASSIMILATION AND ENDOGENOUS PROTEIN SYNTHESIS TO THE TREATMENT OF CARBOHYDRATE WASTES DEFICIENT IN NITROGEN

by

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SUMMARY

A synthetic waste (with glucose as carbon source) devoid of a source of nitrogen was purified in a laboratory scale pilot plant by a new modification of the activated sludge process. The process makes use of a separate carbon assimilation (oxidative assimilation) phase and an endogenous phase in which ammonia is added to a portion of the settled sludge and non-nitrogenous products stored in the cells in the assimilation phase are converted to protein. It was found that sludge so treated could, when recycled to the assimilation tank, carry out continuous oxidative assimilation of the waste. Various COD/N ratios were studied. At the highest, 70/1, ninety per cent purification efficiency was achieved.

INTRODUCTION

The activated sludge process is used extensively for the biological treatment of a wide array of plant effluents containing organic matter. Many wastes are deficient in nitrogen, and this constituent is usually added to the raw waste water at a ratio of approximately one part nitrogen to twenty parts biological oxidation demand (i.e., BOD/N = 20/1). The nitrogen source most commonly employed is anhydrous ammonia, and this supplementation represents a significant portion of the operational costs of the treatment plant. In addition, the operational schemes of some modifications of the activated sludge process are such that a portion of the added nitrogen may pass through the aerator, thus needlessly wasting ammonia and adding to the stream enrichment problem (eutrophication) which is becoming of increasing national concern.

It has been known for some time from studies using both pure cultures and heterogeneous populations of microorganisms that oxidative assimilation of carbon sources can take place in the total absence of an exogenous source of nitrogen (1)(2)(3)(4)(5). From the standpoint of making use of this process as a mechanism of substrate elimination, the initial microbial population would have to be rather large since no net growth (in the sense of cell replication) can take place in such a system. It has been shown that under such nonproliferating conditions, using high initial biological solids concentrations, removal of carbon source and sludge accumulation proceeded, most generally, in accordance with zero order or linear kinetics. However, it has also been shown that the same kinetic order (and almost the same rate) is exhibited in systems containing an ample supply of nitrogen. Recently, it has been shown that the reason for this kinetic similarity in systems which are operationally very different (i.e., growth vs. nonproliferating systems) lies in the fact that at high biological solids concentrations the biological mechanism of substrate removal is very much the same with or without exogenous nitrogen (6)(7). It has been shown that during the period of substrate removal the increase in biological solids, regardless of the presence or absence of a source of nitrogen, is attributable largely to the synthesis of non-protein cellular materials. Thus, at high cell concentrations, exogenous substrate is removed via oxidative assimilation. In systems containing a high biological solids concentration and a nitrogen source, most of the protein synthesis occurs after the synthesis of the non-nitrogenous constituents produced during the period of substrate removal.

The sequential nature of the synthetic processes in growth systems seemed to the authors a fortuitous circumstance which might be usefully exploited from a bioengineering standpoint. It was reasoned that if these processes occurred essentially in a sequential manner when nitrogen was present, it should be feasible to separate the processes physically, i.e., to remove the carbon source from a nitrogen-deficient waste under conditions of oxidative assimilation in the absence of a source of nitrogen, then settle out the sludge, discharge the effluent, and add a source of nitrogen to a portion of the thickened sludge and allow the non-nitrogenous endogenous carbon store synthesized in the nonproliferating phase (or a portion thereof) to be converted to protein. A sludge so treated might be expected to exhibit a regenerated capacity for oxidative assimilation and could be recycled to the oxidative assimilation reactor.

To test this possibility, batch experiments were made in the laboratory using glucose as the carbon source, and the sludge was subjected to the sequential operation outlined above (1). Based upon these studies, it was concluded that the process was mechanistically feasible, and the flow sheet shown in Figure 1 was proposed. Later, in laboratory bench scale studies, the process was successfully employed in continuous flow operation using a sugar refinery waste (8).

The foregoing studies indicated that the proposed process modification held promise, and it was felt that more definitive information pertaining to operational process control and nitrogen requirements could be obtained by performing further continuous flow experiments using a bench scale pilot plant and a chemically-defined nitrogen-deficient synthetic waste.

MATERIALS AND METHODS

A schematic drawing of the experimental pilot plant is shown in Figure 2. Synthetic waste of the following composition was continuously fed to the feeding phase reactor: glucose, 1000 mg/l; 1.0 M potassium phosphate buffer, pH 7.0, 10 ml/l; $MgSO_4 \cdot 7H_2O$, 100 mg/l; $FeCl_3 \cdot 6H_2O$, 0.5 mg/l; $MnSO_4 \cdot H_2O$, 10 mg/l; $CaCl_2$, 7.5 mg/l; tap water, 100 ml/l, and distilled water to volume. A heterogeneous microbial population was developed from a seed inoculum obtained from the effluent of the primary clarifier of the municipal sewage treatment plant at Stillwater, Oklahoma.

The aerated volume of the feeding phase reactor was 2400 ml and the synthetic waste was fed at a rate of 400 ml/hr. The aeration liquor flowed to the settling tank from which the supernatant was continuously discharged. Sludge was harvested from the bottom of the settling tank at 12-hour intervals and a portion of it was diluted to a predetermined optical density

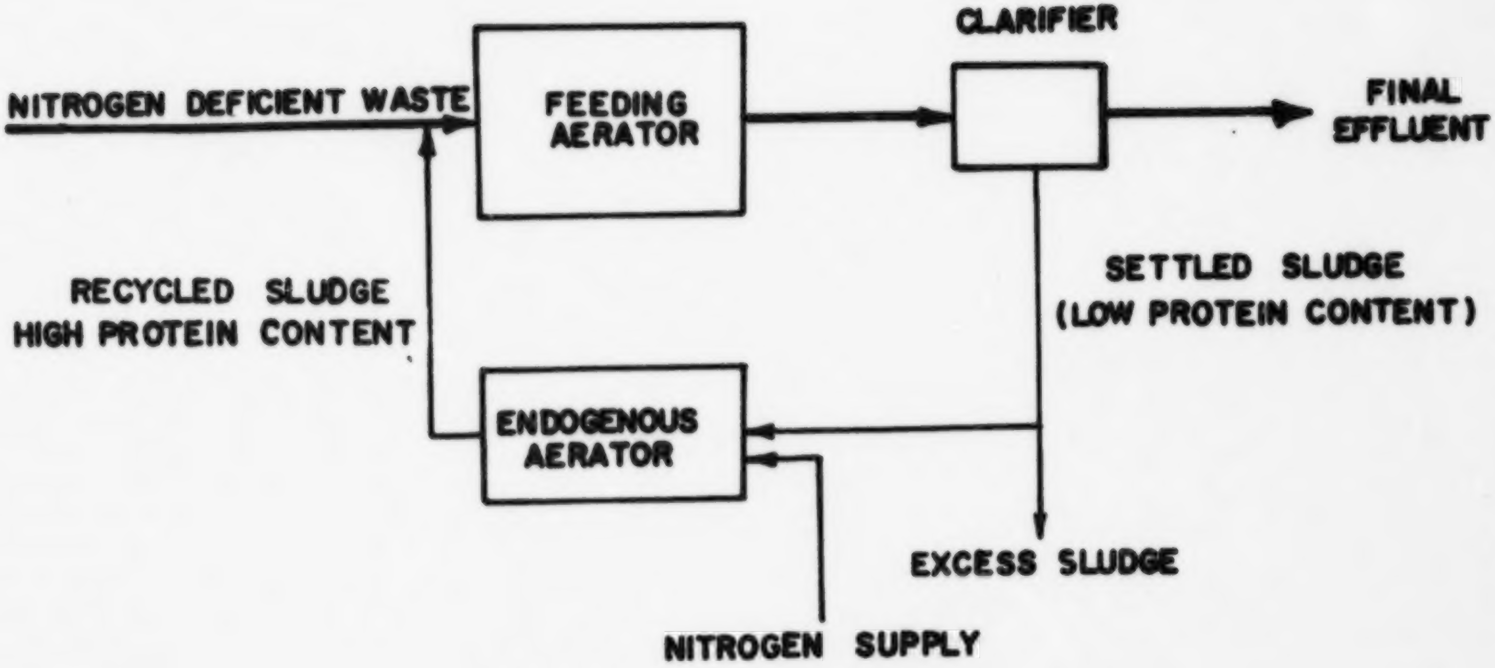

Figure 1 - Process modification for treatment of nitrogen-deficient wastes by activated sludge.

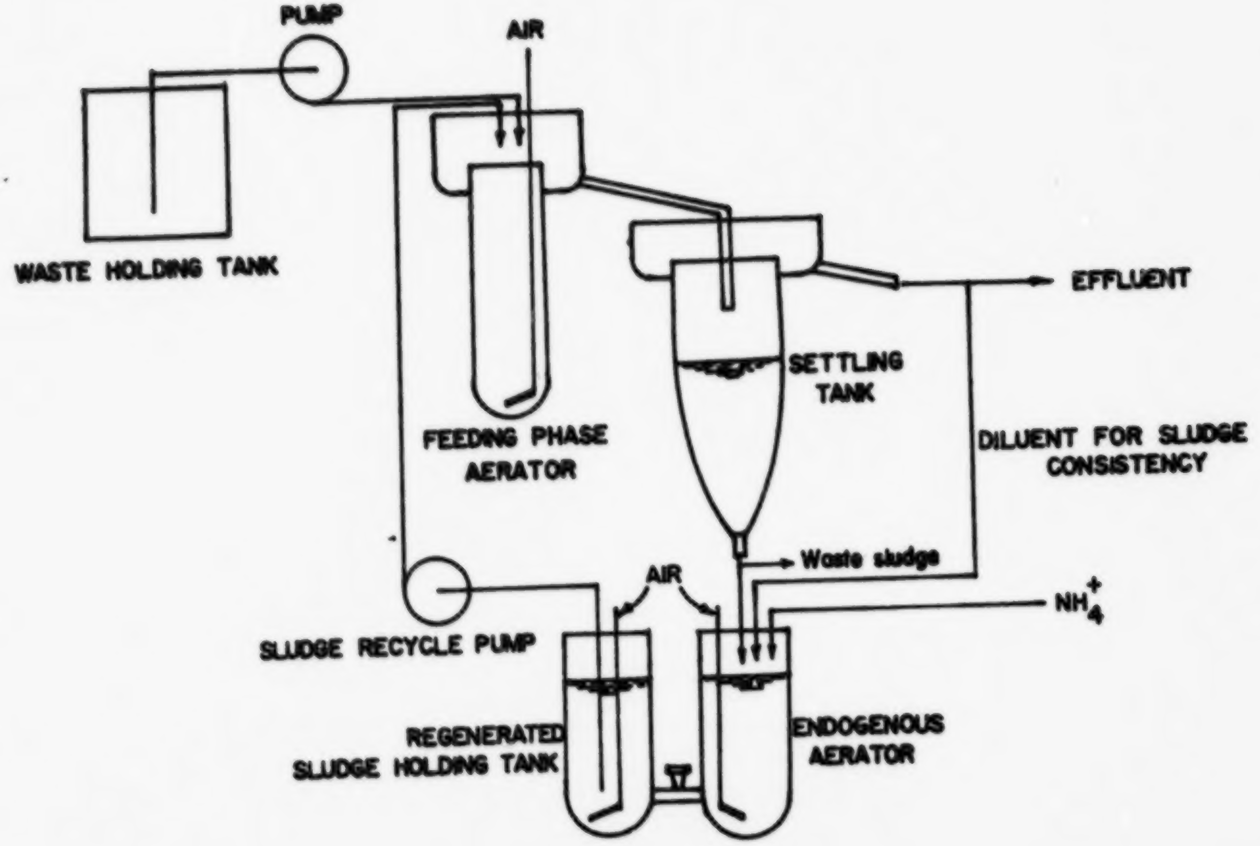

Figure 2 - Laboratory scale pilot plant.

with the supernatant to obtain the desired concentration and volume for intracellular conversion of stored products to protein in the endogenous aerator. The remainder of the sludge was wasted. Ammonium sulfate was added to the portion of the sludge channelled to the endogenous reactor, and the system was aerated for twelve hours. The sludge was then channelled to the regenerated sludge holding tank and pumped to the feeding phase aerator at a rate of 200 ml/hr. The endogenous aerator was then recharged with another batch of sludge which was subjected to regeneration, while the previous batch was being pumped to the feeding phase reactor. Since the total flow to the feeding aerator consisted of 400 ml/hr waste plus 200 ml/hr recycle sludge, the reactor detention time was four hours (i.e., dilution rate, $D = 0.25\ hr^{-1}$). It was felt that employment of this rather short detention time would provide a more critical test of the continuous oxidative assimilation capacity of the system.

In order to compare the nitrogen requirement of this process with the nitrogen supplementation provided for conventional activated sludge processes (i.e., addition of nitrogen to the inflowing waste water), ammonium sulfate was added to the endogenous aerator on the basis of the ratio of chemical oxygen demand to nitrogen (i.e., COD/N ratio). The amount of nitrogen added to the endogenous tank at any desired COD/N ratio was calculated on the basis of the total COD supplied to the feeding aerator each 12-hour period. The theoretical COD of 1000 mg/l glucose is 1060 mg/l (i.e., 192/180 x 1000); this value also checked very closely with values obtained by actual

COD determination. Thus, 5088 mg COD was fed each twelve hours (400 ml/hr x 12 hrs x 1060 mg/l COD). For operation at a COD/N ratio of 10/1, 508.8 mg of NH_3-N were added each time the endogenous aerator was charged with the sludge. This quantity of nitrogen is contained in 2422 mg of ammonium sulfate (508.8/0.21). The volume of sludge added to the endogenous aerator was 2.5 liters; therefore, at a COD/N ratio of 10/1, the concentration of ammonia nitrogen was 203.5 mg/l (508.8/2.5). Similarly, the concentrations of NH_3-N present at the initiation of the endogenous phase at the other COD/N ratios employed, 30/1, 40/1, 50/1, and 70/1 were, respectively, 67.8, 61, 40.7, and 29.1 mg/l.

The pilot plant was run for an extended period of time at each COD/N ratio. Periodically, samples of the settling tank effluent supernatant were analyzed for COD concentration. The aerator mixed liquor effluent was filtered (membrane filter, pore size 0.45 μ) and the COD of the filtrate was determined in order to compare the biochemical and total efficiency of the system (i.e., removal of carbon source only, as compared with carbon source removal plus the flocculation and settling characteristics of the sludge).

Biological solids concentrations in the feeding aerator mixed liquor, as well as in the recycle, were assessed using the membrane filter technique. The protein (biuret) and carbohydrate (anthrone) concentrations of these sludges were also determined (9). Ammonium nitrogen carried over from the endogenous aerator and in the final effluent was determined using the method of Niss, as described by Ecker and Lockhart (10).

RESULTS AND DISCUSSION

The average values for all parameters assessed at each of the five nitrogen supplementation levels are given in Table I. The type of data from which the values in the table were obtained is exemplified by the results shown for the feeding aerator in Figure 3, and for the endogenous aerator in Figure 4.

Before analyzing the results summarized in Table I, it is important to note (Figure 3) that the system operated in a relatively stable manner with respect to effluent COD but was subject to variation in biological solids concentration. Part of this variation was due to operational variation in the concentrations of recycle sludge. The concentration of sludge charged to the endogenous tank was estimated on the basis of optical density and this procedure made it somewhat difficult to reproduce the same sludge concentration in each charge. In Figure 3 the biological solids in the aerator due to recycle is shown (i.e., recycle sludge concentration x 200/600), rather than the actual solids concentration in the recycle flow, in order that the net sludge synthesis (hatched area) due to continuous oxidative assimilation could be shown. In general, the degree of "steady state" behavior which was approached under this mode of operation compares favorably to the "steady state" condition approached for systems operated under the normal mode of operation when employing heterogeneous populations (11). Since the degree of variation in the various parameters was approximately the same for all COD/N ratios, in the interest of brevity only the run made at the 50/1 ratio is shown. Also, the results

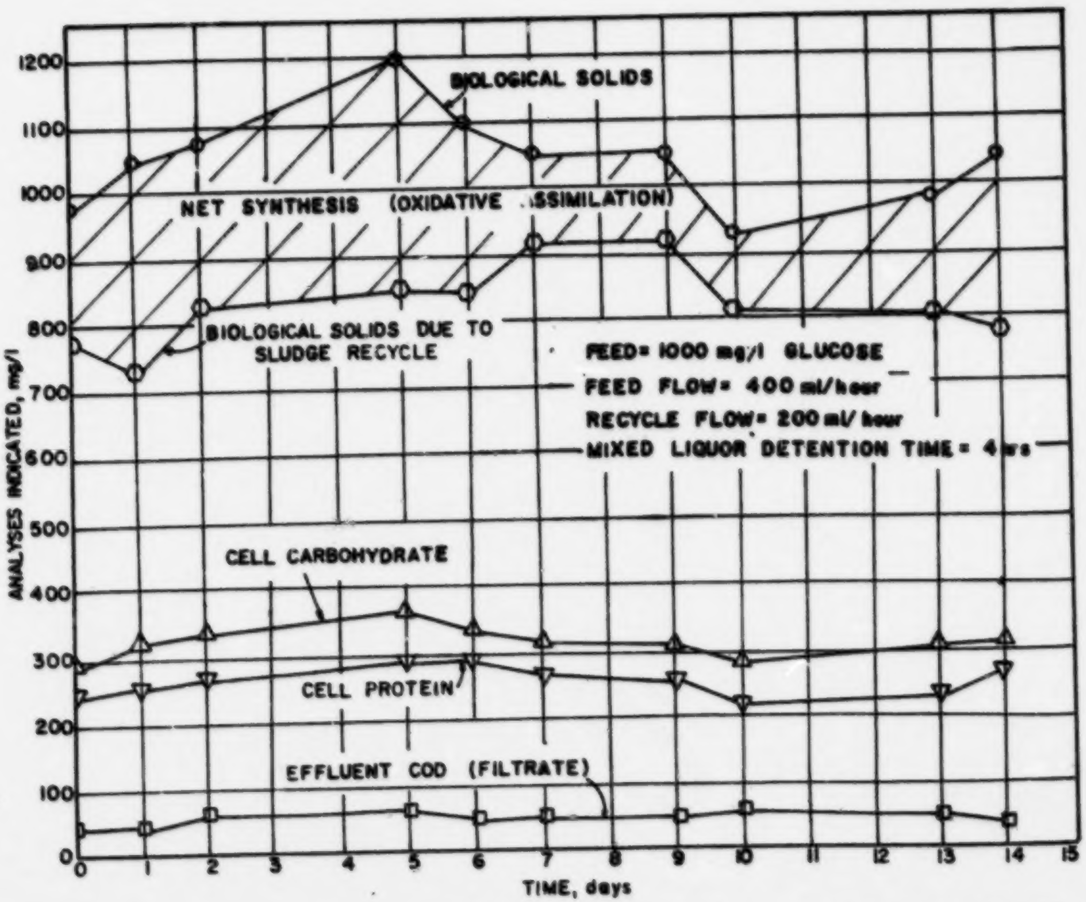

Figure 3 - Continuous oxidative assimilation of glucose in the feeding aerator using biological solids previously subjected to endogenous respiration in the presence of NH_3-N (COD/N = 50/1).

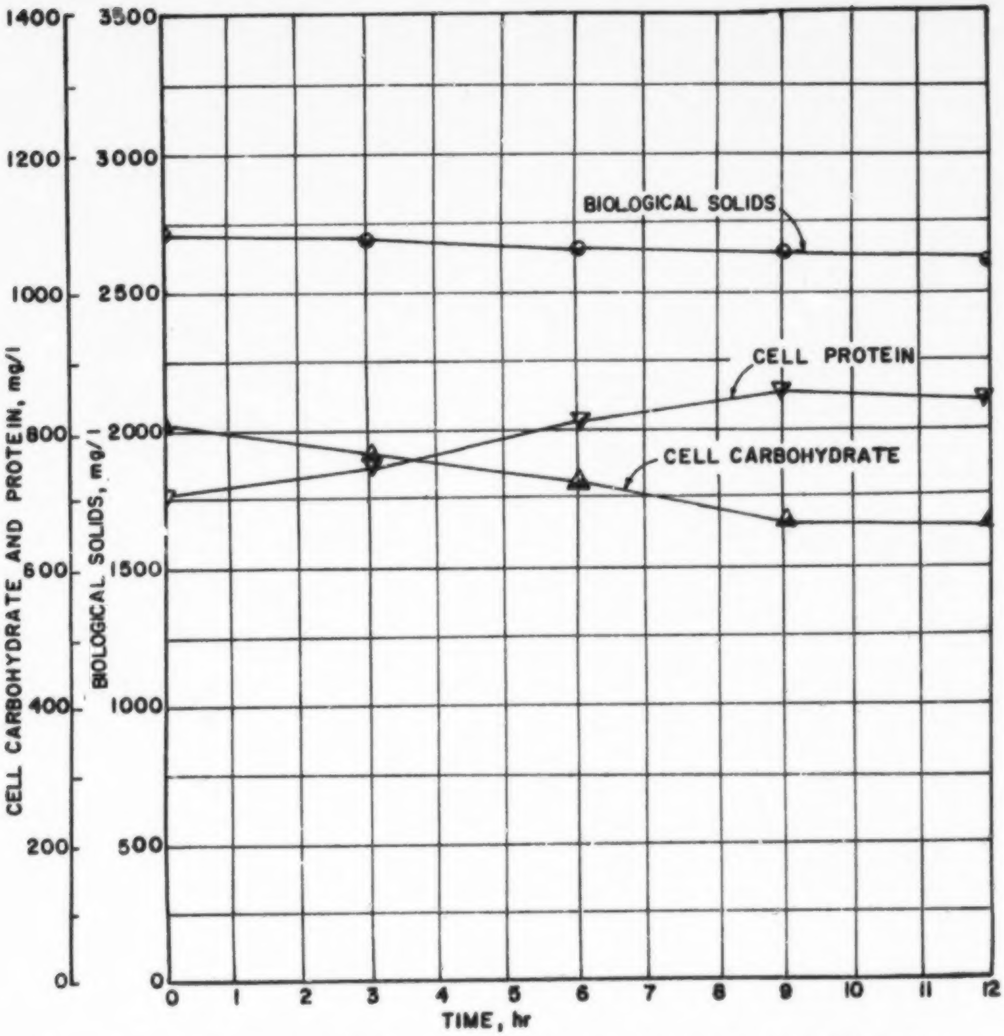

Figure 4 - Intracellular conversion of carbohydrate to protein in the endogenous aerator (COD/N = 50/1).

shown in Figure 4 which depict the kinetics of protein production from the endogenous carbohydrate store, in the presence of an exogenous source of nitrogen, for one of the 12-hour endogenous periods during operation at the COD/N ratio of 50/1 are typical of the many such experiments run during the course of the investigation. It may be seen from Figure 4 that the detention time in the endogenous aerator could have been reduced at least to nine hours without decreased protein synthesis.

From Table I it is seen that excellent removal of carbon source (i.e., waste water purification) was obtained at all COD/N ratios whether measured on the basis of filtrate COD or settled supernatant COD. The columns labeled "filtrate COD" show the values for filtrate COD measured on either the mixed liquor from the feeding phase aerator or the effluent from the aerator, since these values were the same. The column labeled "supernatant COD" represents the COD remaining after quiescent settling of the feeding aerator mixed liquor. Comparison of the effluent quality based on filtrate COD (biochemical efficiency) and that based on supernatant COD (total efficiency) provides an estimate of the flocculation and settling characteristics of the sludge produced under oxidative assimilation conditions. For example, examining the worst case, i.e., at a COD/N ratio of 70/1, the COD in the effluent due to unsettled biological solids was 55 mg/l. It has been determined experimentally that the average COD of 1 mg of cells is 1.37 mg (12). On this basis, the cell concentration in the supernatant was 40 mg/l (i.e., 55/1.37). Since the average sludge concentration

TABLE I

AVERAGE VALUES FOR ANALYSES OF EFFLUENT FROM THE FEEDING AND ENDOGENOUS AERATORS DURING CONTINUOUS OPERATION AT VARIOUS COD/N RATIOS

(D = 0.25 hr^{-1}; Carbon Source = 1000 mg/1 Glucose = 1060 mg/1 COD in the Feed)

	Effluent from Feeding Aerator										Effluent from Endogenous Aerator				
COD:N	Filtrate COD		Supernatant COD (Unfiltered)		NH_3-N in Final Effluent	Sludge	Sludge Yield	Sludge Protein	Sludge Carbohydrate	Sludge Wasted	Carry-over COD	NH_3-N In Carry-Over	Sludge	Sludge Protein	Sludge Carbohydrate
	mg/1	% Removal	mg/1	% Removal	mg/1	mg/1	%	%	%	%	mg/1	mg/1	mg/1	%	%
10:1	32	97	60	94	25	1600	80	28	22	34	43	160	3160	31	20
30:1	34	97	62	94	1	1940	82	28	24	29	43	45	4130	31	20
40:1	78	93	105	90	0	1520	70	26	25	30	86	34	3190	29	20
50:1	50	95	102	90	0	1046	32	24	30	21	65	5	2480	32	25
70:1	50	95	105	90	0	1007	26	24	30	17	60	0	2490	31	25

in the aerator mixed liquor was 1007 mg/l, the flocculation and settling ability of the sludge can be calculated to be approximately 96 per cent. Also, it should be noted that the sludge settled rapidly and exhibited no bulking or rising tendency throughout the investigational period. However, as with any other type of activated sludge process, there is no guarantee that flocculation and settling (and lack of bulking or rising sludge) would always be as good as was observed in these studies. While it should not be surmised that the sludge produced by this continuous oxidative assimilation process possesses superior flocculation and settling characteristics, it can be stated on the basis of the present results that it possesses characteristics as good as those of conventionally operated activated sludge processes.

The effluent was essentially devoid of ammonia nitrogen when the COD/N ratio was maintained at 30/1 or above. However, even at a COD/N ratio of 50/1, not all of the nitrogen was used in the endogenous aerator. At this ratio approximately 1.8 mg/l NH_3-N (i.e., $5 \times \frac{200}{600}$) was fed into the feeding aerator due to cell recycle. This is a very small amount of nitrogen, and in any event, there is no doubt that strict nonproliferating conditions were maintained in the feeding aerator during operation at a COD/N ratio of 70/1.

It is seen from Table I that the COD/N ratio exerted a significant effect on the sludge (cell) yield. The cell yield was calculated as the ratio of the hourly rate of sludge production (outflow rate minus inflow rate) to the hourly rate of

COD consumption (inflow rate in feed and recycle minus outflow rate). Sample calculations are given below for operation at the COD/N ratio of 30/1.

Rate of Sludge Production:

Sludge output = 1940 mg/l x 0.6 l/hr = 1164 mg/hr
Sludge input = 4130 mg/l x 0.2 l/hr = 826 mg/hr
Net sludge produced = 1164 - 826 = 338 mg/hr

Rate of COD Consumption:

COD input (feed) = 1060 mg/l x 0.4 l/hr = 424 mg/hr
COD input (recycle) = 43 mg/l x 0.2 l/hr = 9 mg/hr
COD input (total) = 424 + 9 = 433 mg/hr
COD output = 34 mg/l x 0.6 l/hr = 20 mg/hr
COD consumed = 433 - 20 = 413 mg/hr

Sludge yield = 338/413 = 0.82.

Rather high sludge yields were obtained at the three highest nitrogen supplementation levels, but yield decreased rather sharply as the COD/N ratio was increased. Since the COD removal efficiency of the system remained high, it would appear that the amount of substrate oxidized (respired) may be expected to increase as the COD/N ratio is increased. Such a situation would seem advantageous from the standpoint of sludge disposal, since it is seen from Table I that a greater percentage of the synthesized sludge was returned to the aerator (i.e., less sludge was wasted) at the highest COD/N ratio in order to maintain a high level of aeration solids in the feeding aerator.

With regard to the relative increase in protein and decrease in carbohydrate which took place in the endogenous aerator, it may be discerned that the interconversion of stored products to protein (change in percent composition) was greatest for the systems operated at the COD/N ratios of 50/1 and 70/1 which were the systems for which it can be stated unequivocally that nonproliferating conditions existed in the feeding phase aerator.

In order to facilitate comparison of the various operational parameters at the five levels of nitrogen supplementation in the endogenous aerator and to estimate their values at intermediate levels, the results are plotted in Figure 5. This figure shows at a glance the fact that the most drastic effect was on the sludge yield and that the most drastic change in sludge yield occurred between COD/N ratios of 40/1 and 50/1.

There is much yet to be learned about this proposed modification to the activated sludge process for treatment of nitrogen-deficient wastes. However, the results clearly indicate that continuous oxidative assimilation, made possible by conversion of the intracellular storage products to nitrogenous cell constituents in a separate phase (endogenous with respect to carbon source) in the presence of an exogenous source of nitrogen and subsequent recycling of the sludge to the assimilation tank, provides a feasible wastewater treatment process. Furthermore, the process can be run without leakage of nitrogen (NH_3-N) in the effluent. In the present study the lowest total purification efficiency was ninety per cent, and this degree of purification was attainable at the rather high COD/N ratio of 70/1. In the present study, COD rather than biochemical oxygen

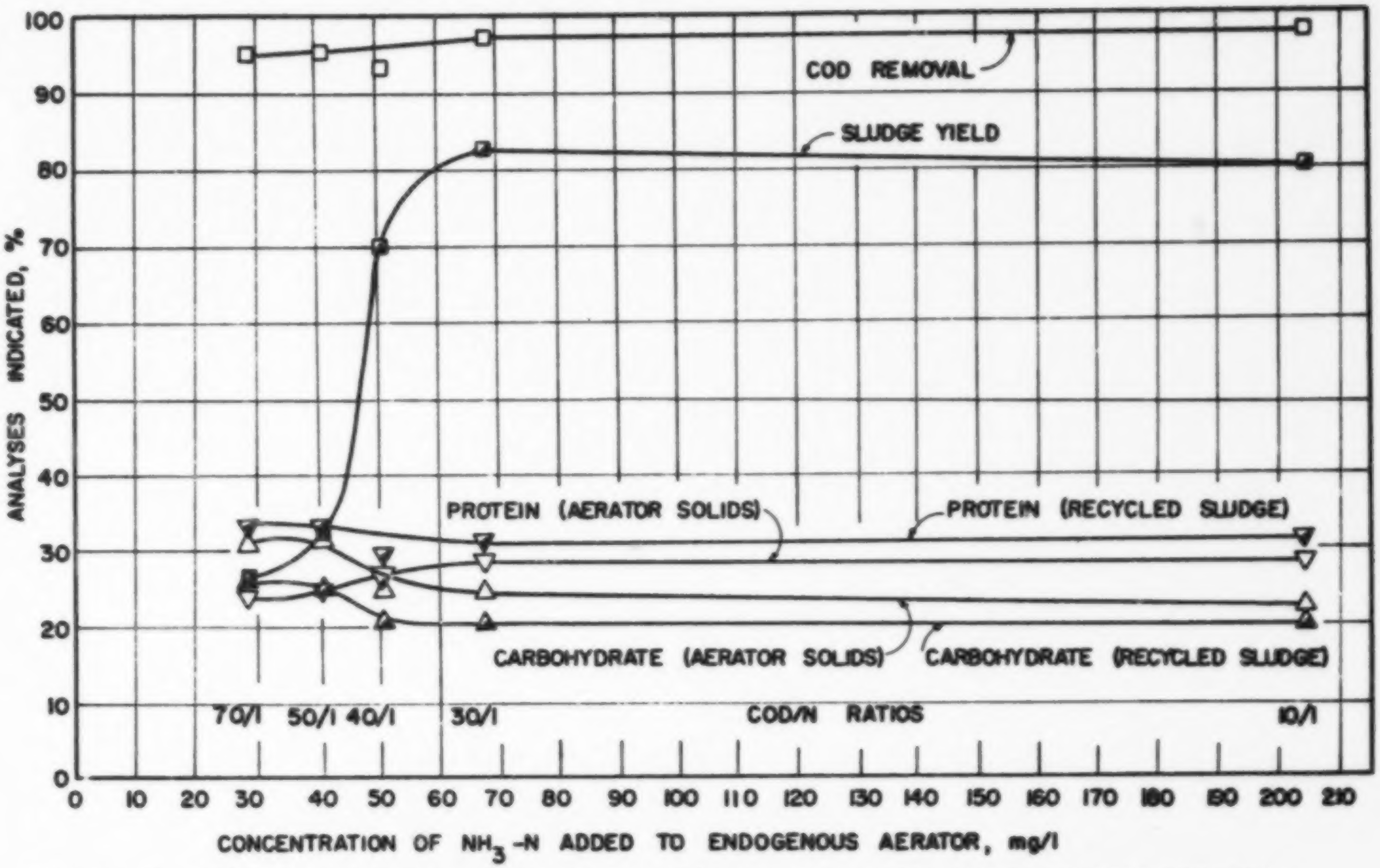

Figure 5 - Effect of endogenous phase nitrogen concentration on COD removal, sludge yield, and cell composition.

demand (BOD) was used as the operational parameter for measuring the efficiency of purification because this measurement is widely used in control of industrial waste treatment plant operation. For the carbon source herein employed, the loadings and the COD/N ratios can be easily converted to the BOD base. For example, the BOD/COD ratio for glucose was determined experimentally to be 0.7. Therefore, the COD/N ratio of 70/1 represented a BOD/N ratio of 49/1. Since the BOD/N ratio commonly employed is 20/1, it is evident that a significant savings in nitrogen was effected, with the additional advantage that ammonia did not escape in the plant effluent.

ACKNOWLEDGEMENT

This work was supported by a Research Grant WP 00325 from the Water Pollution Control Administration, United States Department of the Interior.

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PAPER 24

Rickard, M. D., and Gaudy, A. F. Jr., "Effect of Mixing Energy on Sludge Yield and Cell Composition for Heterogeneous Populations in Completely Mixed Systems."

This paper was presented at the 39th Annual Conference of the Water Pollution Control Federation, New York City, New York, October, 1967. Journal Water Pollution Control Federation (in Press) 1968.

EFFECT OF MIXING ENERGY ON SLUDGE YIELD AND CELL COMPOSITION FOR HETEROGENEOUS POPULATIONS IN COMPLETELY MIXED SYSTEMS

By

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INTRODUCTION

The effect of oxygen tension on the growth and respiration of both pure and heterogeneous bacterial cultures has been of considerable interest to both microbiologists and sanitary engineers. As a result, a good understanding of the dissolved oxygen requirements of microbial systems has been developed. Bennet and Kempe (1) have reviewed the literature concerning the mechanisms involved in the transfer of oxygen to the bacterial cell and concluded that, for a given culture, a critical value of dissolved oxygen exists above which no increase in the rate of respiration will be observed with increasing DO. The critical values reported by these authors ranged from 0.1 to 1.53 mg/l of dissolved oxygen for the various pure cultures that have been studied, and 1.0 to 2.0 mg/l for activated sludge.

Presented at the 39th Annual Conference of the Water Pollution Control Federation, New York, New York, October, 1967.

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In contrast, the effect of the agitation provided to a bacterial system has received scant attention by workers in this country. Tsao and Kempe (2) observed that an increase in the oxygen uptake of Pseudomonas ovalis occurred as the turbulence of the system was increased by increasing the rate of mechanical stirring. Rincke (3), citing the work of Imhoff (4) and von der Emde (5), further stated that the rates of oxygen uptake and substrate utilization increased in activated sludge with increases in the rate of mixing.

Pasveer (6), in his study of brush aeration, found that the purification ability of activated sludge increased as the oxygenation capacity of the aeration unit was increased. Although he expressed his results in terms of oxygenating capacity, Pasveer attributed the effects observed to a reduction in floc size, induced by mechanical shear, at the high oxygenation rates.

Zahradka (7) determined that a definite relation existed between the mechanical energy supplied to an activated sludge pilot plant and the purifying ability of the sludge. He was also able to demonstrate that increasing energy input to the system resulted in a shift in the population dynamics of his system away from the predominance of filamentous organisms. As a result of this work, he concluded that the BOD loading per unit of mechanical energy supplied is a better parameter for design and operation of activated sludge than is the BOD to solids loading.

Bennet and Kempe (1) have observed that the rate of gluconic acid production by a resting suspension of P.

ovalis aerobically metabolizing glucose increased when the oxygen tension of the medium was elevated by raising either the air flow rate or the agitation rate of their fermenter. This increase was observed even though the dissolved oxygen concentration of the medium was above the critical level for the organism used. Considering the information presented previously, it is possible that the behavior of Bennet and Kempe's system could be explained as being caused by increased turbulence, since the amount of mechanical energy added would change with changes in either the rate of agitation or aeration.

In spite of the obvious importance of mixing as a parameter in activated sludge, surprisingly little is known about the response of bacterial systems to changes in the amount of turbulence provided to the medium. The investigation reported herein was made in order to gain a more complete understanding of the effect of agitation on the growth and substrate utilization of continuous flow heterogeneous bacterial systems.

THEORETICAL CONSIDERATIONS

Perhaps the most convenient method of expressing the mechanical energy input to a fluid system is the mean velocity gradient imparted to the fluid mass. Fair and Geyer (8) have presented equations which allow the velocity gradient to be determined for systems agitated by either mechanical stirring or by bubble aeration.

Zahradka (7) has considered further the problem of determination of velocity gradient in systems agitated by diffused air. His development is based on a number of factors including bubble size, type of movement, and distribution of the bubbles leaving the aerator. Unfortunately, the difficulty involved in evaluating some of the factors included in these equations severely limits their utility. As it was not the purpose of this paper to evaluate the various models that have been proposed for determination of velocity gradient, only a brief discussion is included. Interested readers are referred to the original work cited above and to that of Camp and Stein (9) and Camp (10) for a more complete development.

For the purpose of this investigation the equations presented by Fair and Geyer (8) have been used, as these expressions are easily evaluated for a system of the type herein described. According to these relationships, the average velocity gradient imparted to a fluid mass is a function of the power dissipation in the system, the viscosity of the medium, and the volume of fluid involved. Thus, for a stirred system the velocity gradient, G, is given by

$$G = \sqrt{C_d A v^3 / 2\mu V} \qquad (1)$$

where C_d is the drag coefficient of the impeller, A is the total impeller area, v is the velocity of the impeller, μ is the viscosity of the medium, and V is the volume of fluid. For a vessel agitated by bubble aeration, assuming isothermal expansion of the rising bubble

$$G = \sqrt{\frac{81.5\, Q_a \log (H+34)/34}{\mu V}} \qquad (2)$$

where Q_a is the air flow rate, and H is the depth of the liquid in the reactor. In a reactor agitated by both stirring and bubble aeration, the velocity gradient may be determined by addition of the two components defined by equations (1) and (2).

Using the volume and depth of the fluid for the reactor employed in the present studies in these equations and assuming a value for the drag coefficient of 1.2 [from Rouse (11)] and a viscosity equal to that of water at 30°C., the following expression for the velocity gradient in ft/sec/ft (or sec^{-1}) is obtained

$$G = 0.109\, R^{3/2} + 111.6\, A^{1/2} \qquad (3)$$

where R is the speed of the impeller in RPM, and A is the air flow rate in liters/min.

The mixing energy imparted to a liquid bacterial culture has been proposed to affect the growth and purification ability of the bacterial cells in a number of ways. Tsao and Kempe (2) proposed that the rate-limiting step for oxygen uptake in systems containing a high DO occurs in the transfer of oxygen across the cell-liquid interface. They showed that, if this is the case, the rate of oxygen transfer into the cell, and hence the rate of oxygen uptake, should be a function of the fluid properties of the system, the gas flow rate, the rate of agitation, and the physical configuration of the agitator, and not of the oxygen deficit. Thus, this development predicts that the oxygen uptake of a bacterial system is independent of the dissolved oxygen content of the medium, and it is therefore compatible with the critical DO concept. Swilley, Bryant, and Busch (12), on the other hand, agree as to the importance of cell-liquid interfacial transfer rate, but from a consideration of the principles of mass transfer prefer a first order model.

In contrast to the above, Rincke (3) attributes the improved performance of activated sludge at high turbulence simply to improved frequency of contact between the cells and substrate, while Pasveer (6) and Zahradka (7) concluded that the smaller flocs produced at high mechanical energy inputs are the important factor.

In order to establish the general range of values of mechanical energy inputs that might practically be applied to activated sludge, the velocity gradient has been determined for a conventional diffused air plant having the following characteristics: design flow, 1×10^6 gal/day; BOD applied to the aerator, 1000 mg/l; detention time, 6 hrs.; aerator depth, 12 ft.; and design air flow rate, 1500 ft^3/# BOD/day. Based on Equation (2), this plant would furnish an average velocity gradient of 365 sec^{-1} to the mixed liquor.

EXPERIMENTAL METHODS

Experimental Unit

A 5-liter Microferm laboratory fermenter (New Brunswick Scientific Co.) was used throughout these studies. Aeration of the medium was provided by a single air outlet in the bottom of the culture vessel through which filtered compressed air was passed. Although designed for batch operation, the unit was converted to continuous flow operation by the addition of an influent feed line and an inverted standpipe, positive suction, effluent line. Sigmamotor model AL4E pumps were used on both the influent and effluent lines. A schematic diagram of the unit as used is shown in Figure 1.

As the volume of the medium in the culture vessel was found to change with varying agitation rate, the volume actually contained during operation was determined for a number of agitation rates over the range to be used in the experiments. This was accomplished by filling the reactor with a premeasured amount of tap water, setting the agitation and aeration at the desired level, and pumping the excess water above the standpipe level into a graduated cylinder. The volume of the reactor under the test conditions could then be determined by subtraction. The effect of agitation on the volume of medium maintained under aeration at an air flow rate of 1.0 liter/min is shown in Figure 2. Once these data had been determined, it was possible to calculate the necessary flow rate to obtain a

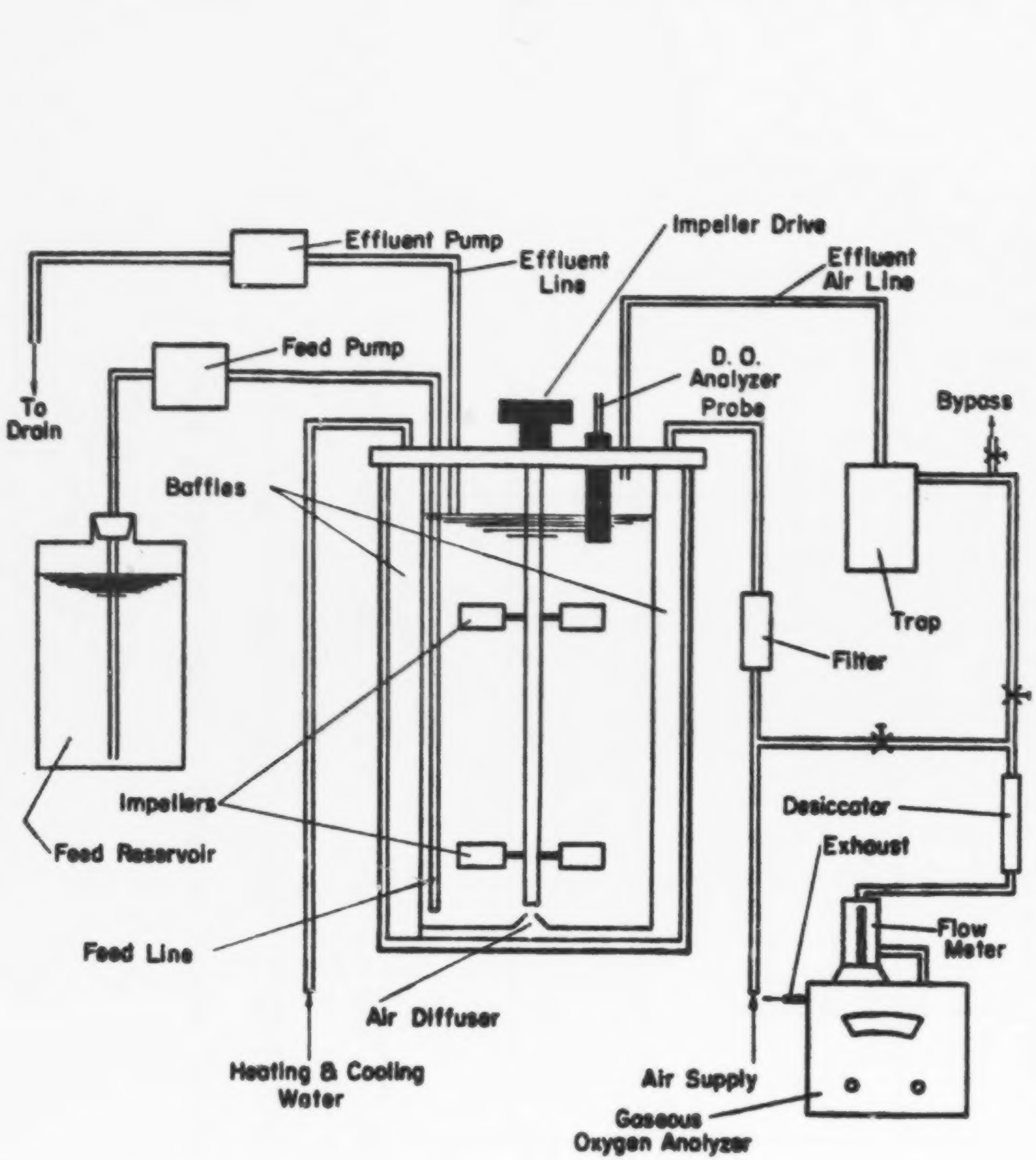

Fig. 1 Schematic diagram of experimental unit.

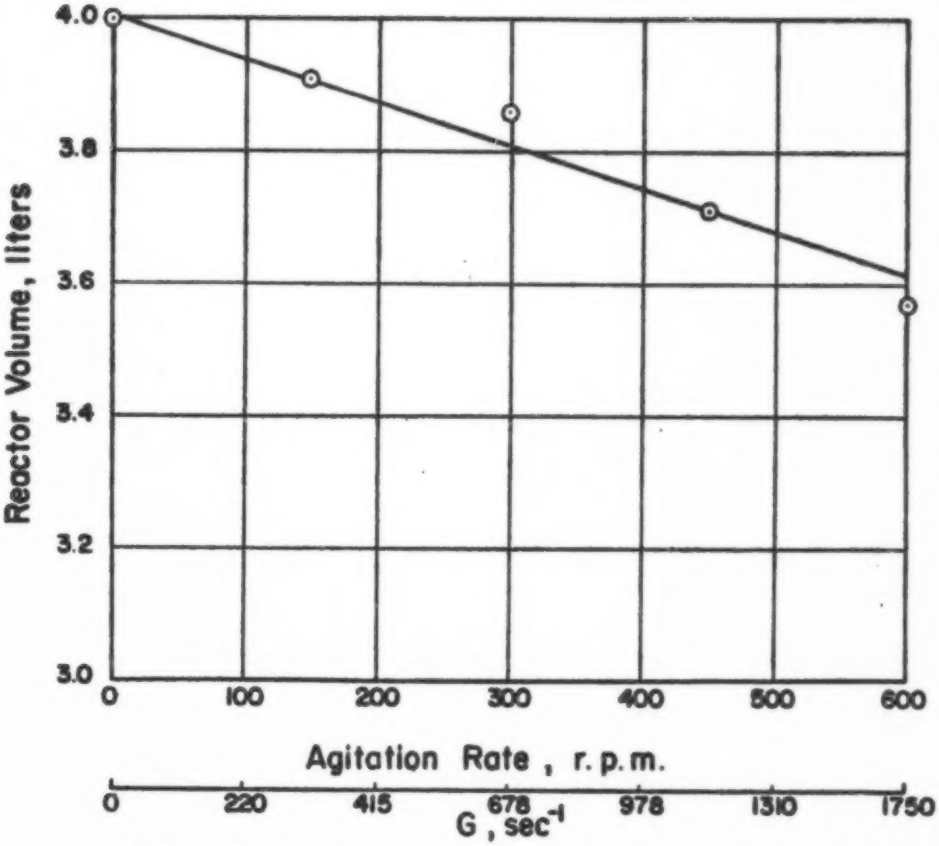

Fig. 2 Effect of Agitation Rate on Reactor Volume.

constant detention time so that all experiments could be conducted at exactly the same dilution rate.

In order to ensure that the unit was completely mixed, dilute-out curves were determined for a series of agitation rates covering the entire range of velocity gradients to be used. These experiments were accomplished by filling the culture vessel with tap water containing phenol at 1000 mg/l COD and diluting out with tap water at a flow rate designed to give a detention time of eight hours. The effluent COD was then determined at appropriate times, and the dilute-out curve plotted. These data could then be compared statistically with the theoretical dilute-out equation assuming complete mixing

$$\ln (C/C_o) = Dt \qquad (4)$$

where D is the dilute-out rate, C_o is the initial COD, and C is the COD in the effluent at time t. At all of the agitation rates examined, the data agreed with the theoretical relationship at the 99% confidence level. The theoretical dilute-out for the reactor at the 8-hour detention time ($D = 0.125$) and the experimental points determined at four different agitation rates are shown in Figure 3.

Since the oxygen transfer rate would be expected to vary with the agitation rate of the medium, it was considered necessary to define this relationship for the experimental system employed. The rate of absorption of oxygen was measured at a number of agitation rates by stripping all of the DO from the medium with nitrogen, and following the

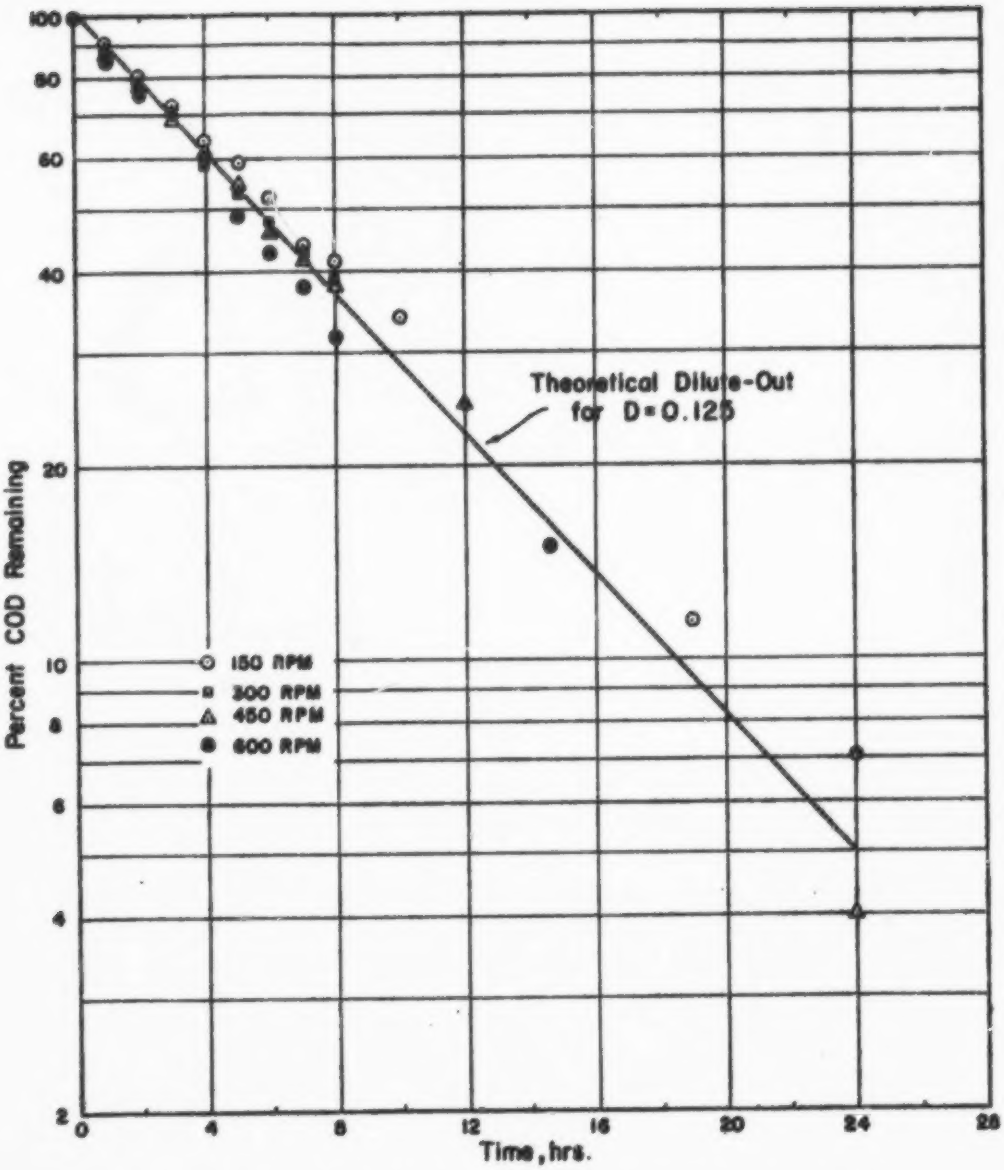

Fig. 3 Comparison of Experimental and Theoretical Dilute-Out Curves at D=0.125 .

increase in dissolved oxygen during reaeration at an air flow rate of 1.0 liter/min. The reaeration coefficient, K_a, was calculated for each run by the method described by Isaacs and Gaudy (13). Regression analysis was then applied to the values obtained for each agitation rate to determine a functional relationship between agitation and K_a

Bacterial Medium

The medium used in these experiments consisted of a glucose mineral salts solution having the composition shown in Table I. The adequacy of the buffer system used was demonstrated by checking the pH of a number of effluent samples during steady state operation. These values were found to be 6.7 ± 0.1.

Experimental Protocol and Analytical Methods

The experimental unit was started by filling the reactor vessel with the standard glucose medium and adding 100 ml of primary effluent from the Stillwater, Oklahoma, municipal waste treatment plant. Operation was then conducted in a "batch" manner for twenty-four hours at 30°C. to allow the development of a vigorous bacterial population. Pumping was then started and the unit operated under continuous flow conditions for seventy-two hours before an experiment was started. At the conclusion of each run, the velocity gradient was changed to the next desired value and the unit operated for twenty-four hours. Operation was then halted, and the culture vessel was inspected for growth adhering to the vessel or baffles. Any such growth was

Table I

Composition of Minimal Medium

Component	Concentration
Glucose	1000 mg/l.
$(NH_4)_2SO_4$	500 mg/l.
$MgSO_4$	200 mg/l.
$MnSO_4$	20 mg/l.
$CaCl_2$	15 mg/l.
$FeCl_3$	1 mg/l.
Tap Water	100 ml/l.
1 M. Phosphate Buffer	15 ml/l.

removed and operation continued for an additional twenty-four hours. During this latter period, the optical density of a number of samples was determined to insure that the unit had achieved steady state prior to the initiation of sampling. In all cases the unit was found to achieve steady state with respect to the optical density during the twenty-four hour period.

Seven experiments were conducted at velocity gradients ranging from 310 to 1350 sec^{-1}, a detention time of eight hours ($D = 0.125$), a temperature of 30°C. ± 0.25°C., and an air flow rate of 1.0 liter/min. The solids level maintained in the reactor during steady state growth was determined by the membrane filter technique. Substrate utilization was determined by analyzing the membrane filtrate for both COD (14) and total carbohydrate (15). The influent COD and glucose concentration were also determined so that percentage removals in terms of both parameters could be calculated.

Samples for cell composition analysis were taken by the method outlined by Gaudy (15). This resulted in a washed cell suspension having ten times the solids concentration of the culture sampled. One ml. of this suspension was subjected to hot perchloric acid extraction according to the method of Burton (16) for subsequent nucleic acid analysis. The remainder (4 ml.) was frozen and stored for carbohydrate and protein determination.

RNA analyses were performed on the perchloric acid extract according to the orcinol method reported by

Ceriotti (17), except that extraction of the final colored product with isoamyl alcohol to achieve concentration of the color was eliminated as it was shown to be unnecessary with the concentrations found in the samples obtained.

DNA was determined on the perchloric acid extract by the diphenylamine reaction as given by Burton (16). Cellular carbohydrates and protein were determined on the washed cell suspension by the anthrone and biuret methods, respectively (15) The dissolved oxygen content of the medium was determined continuously during steady state operation by a galvanic cell dissolved oxygen analyzer (Precision Scientific Co.) connected to a 10 mv recorder (E. H. Sargent Co.).

Oxygen uptake during steady state growth was determined by measuring the difference in oxygen content of the influent and effluent gas stream, in accordance with the manufacturers' recommendations (18) using a Beckman Model E2 gaseous oxygen analyzer. This instrument allowed the determination of the oxygen content of the gas stream with a precision of 0.01% in the range used. This difference was then converted to mg. of oxygen consumed per hour, and the values divided by the volume of the medium in the fermenter under the conditions employed (Figure 2) to obtain the rate of oxygen uptake in mg/liter/hr.

RESULTS

The variation in the oxygen transfer rate of the system as a function of the agitator speed, R, is shown in

Figure 4. As expected, the oxygen transfer rate constant, K_a, increased with increasing agitation of the medium. The least squares line of best fit for the unit employed in these studies is given by

$$\log (100\ K_a) = 1.62 \log R - 2.58 \qquad (5)$$

Figures 5 and 6 show the results of all of the analyses performed on the system for the experimental run at a velocity gradient of 1010 ft/sec/ft. Since it was desired to have a single value representative of each of these parameters at each velocity gradient, and since some variation in the steady state values was observed, a minimum of seven observations of each parameter was made during each run and the average value calculated.

The average steady state dissolved oxygen concentrations observed for experiments in which agitation rate was varied are shown in Figure 7. Since the organic loading, detention time, and air flow rate were held constant, the increase in dissolved oxygen concentration with increasing velocity gradient is a direct reflection of the higher K_a values which occurred at the higher agitation rates.

The average values for the cell composition and biological solids concentration for each run are plotted versus the velocity gradient in Figure 8. In order that these results could be compared directly, the yields of the cellular components were calculated by dividing the concentration of material observed by the difference between the influent and effluent COD. Figure 9 is a plot

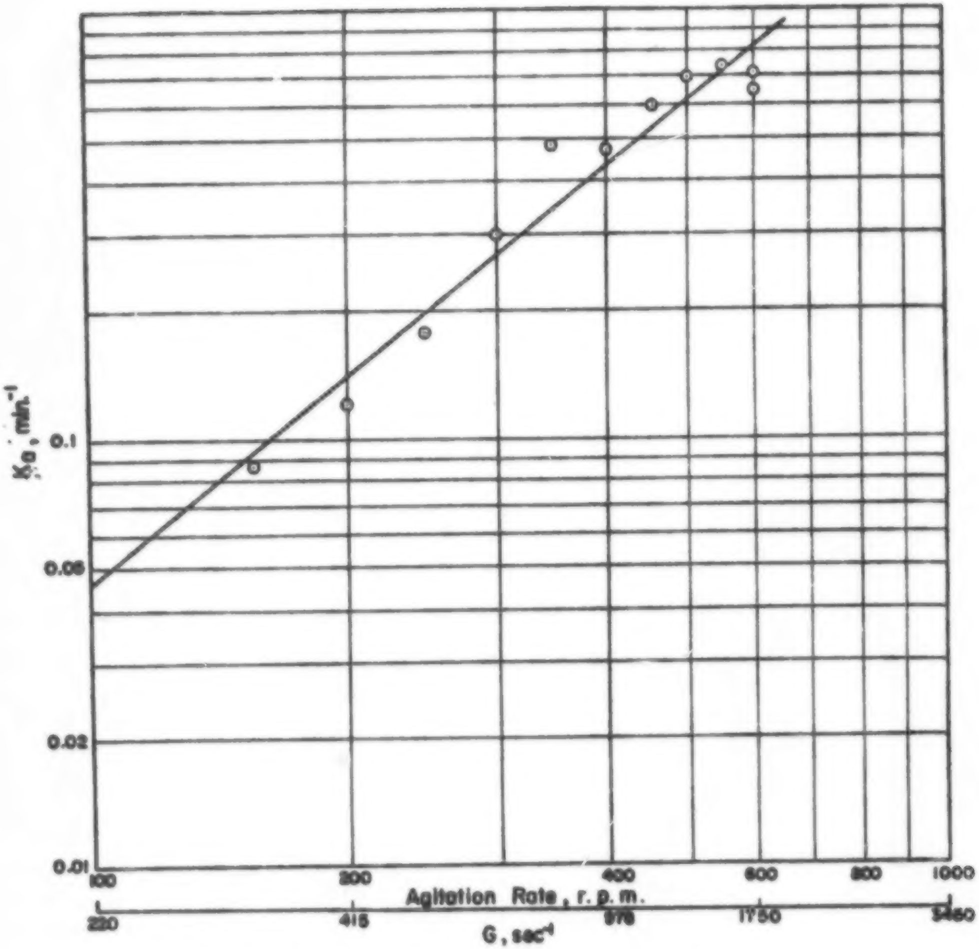

Fig. 4. Variation in Reaeration Coefficient with Changing Agitation Rate.

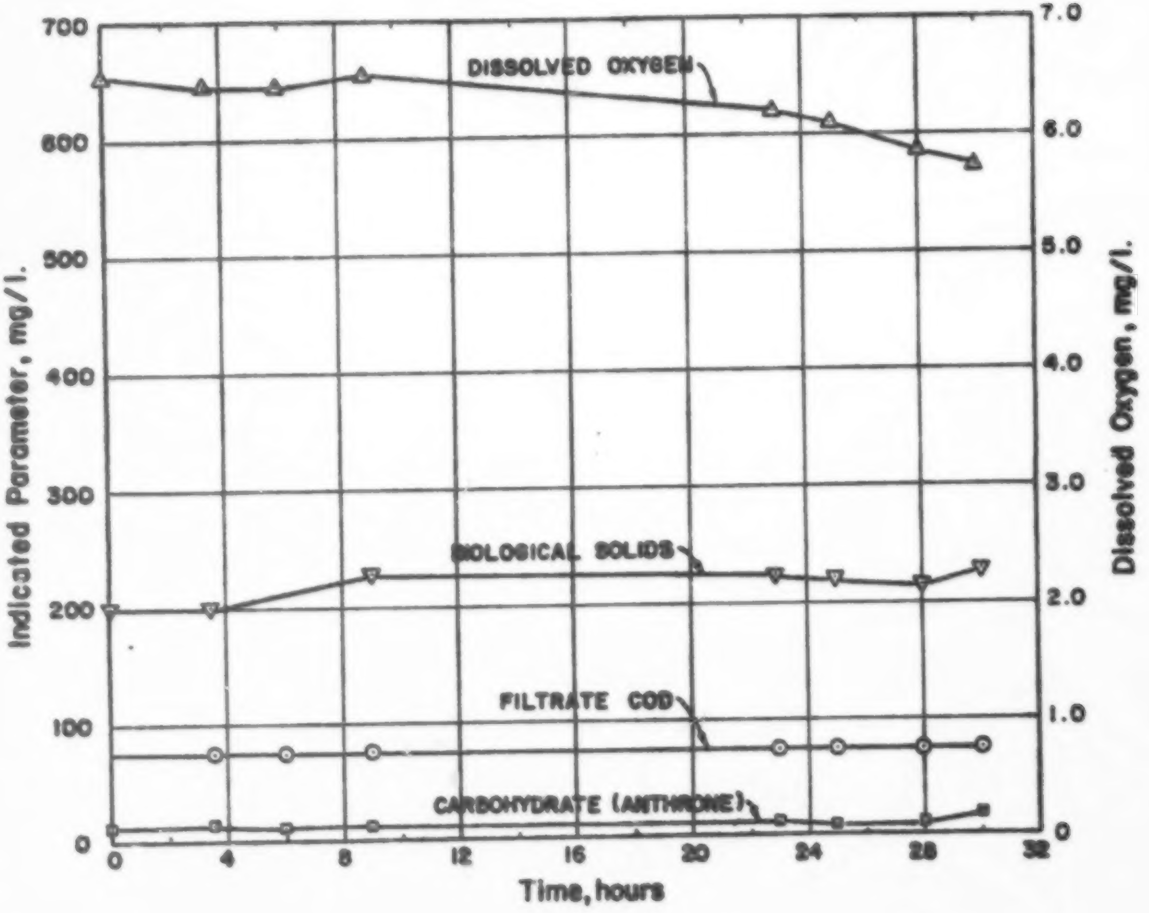

Fig. 5 Steady State Parameters in the Completely Mixed Reactor at a Velocity Gradient of 1010 sec^{-1}.

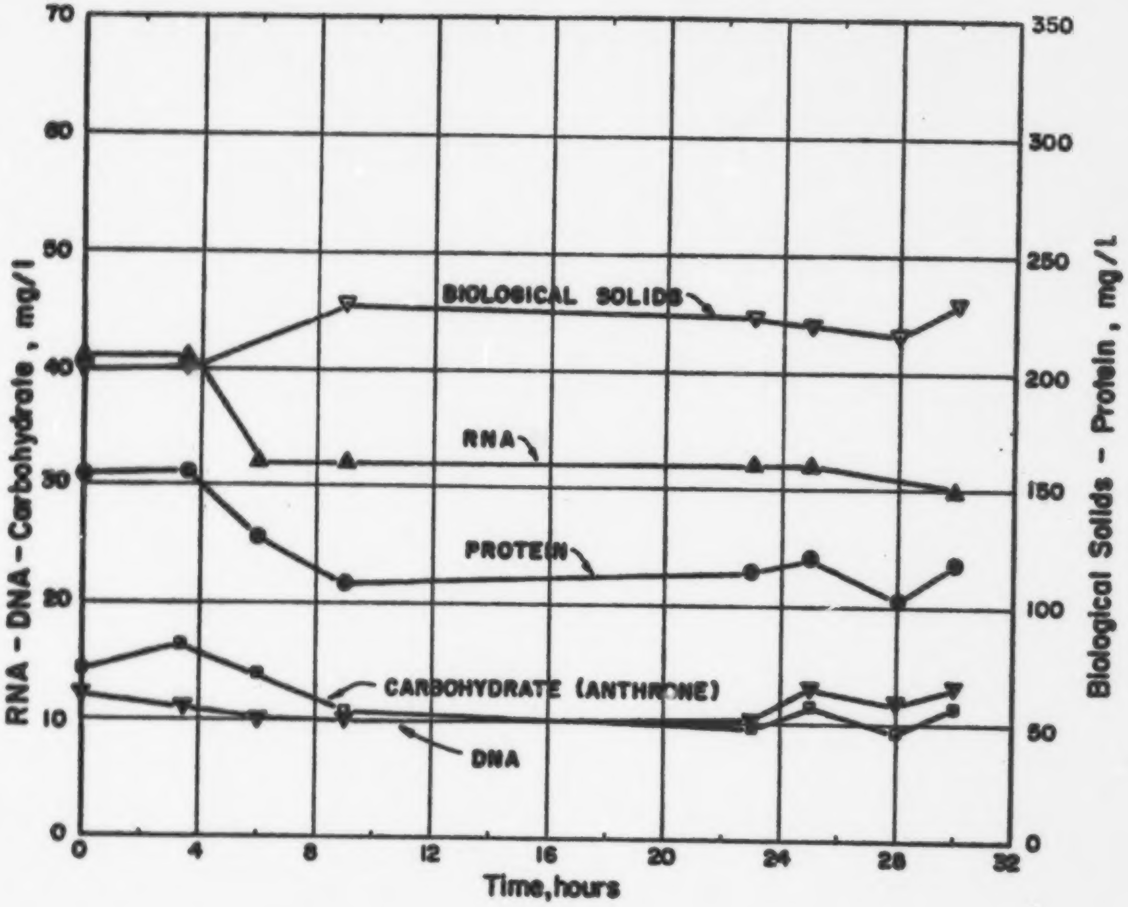

Fig. 6 Steady State Cell Composition in the Completely Mixed Reactor at a Velocity Gradient of 1010 sec^{-1} .

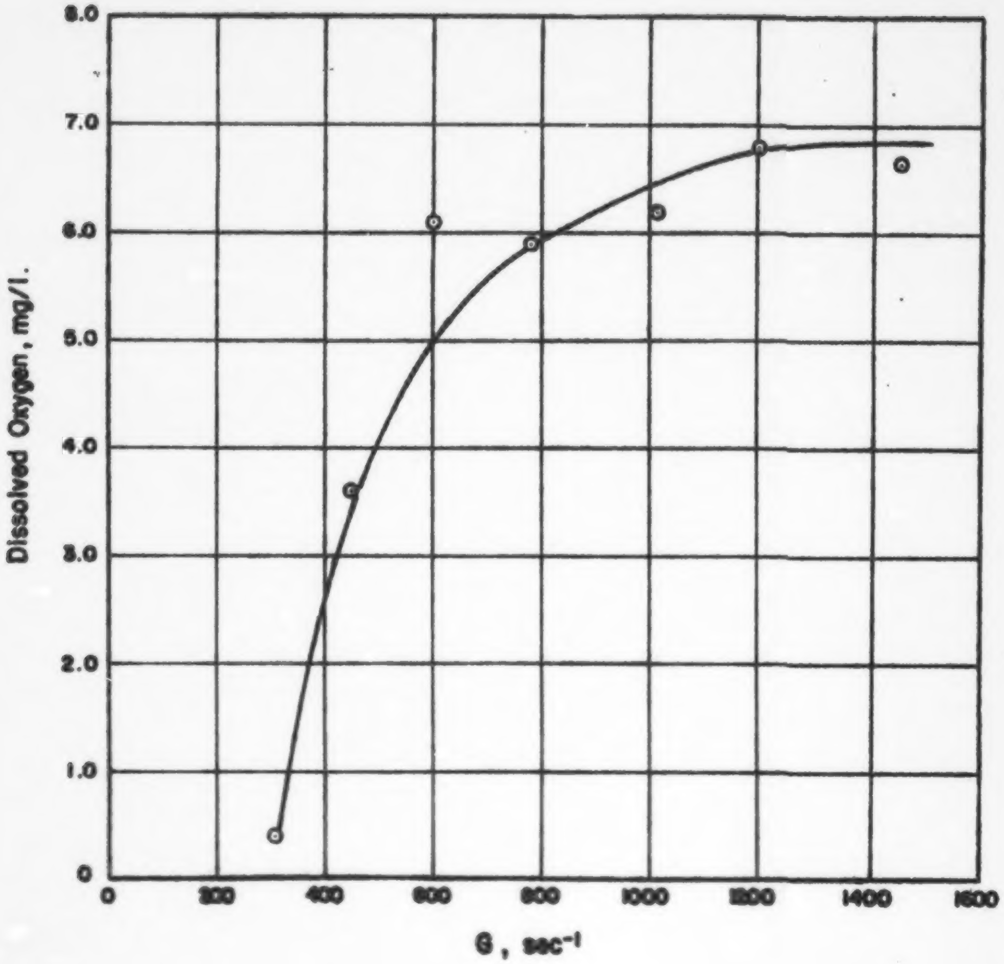

Fig. 7 Relationship between Steady State Dissolved Oxygen and Velocity Gradient.

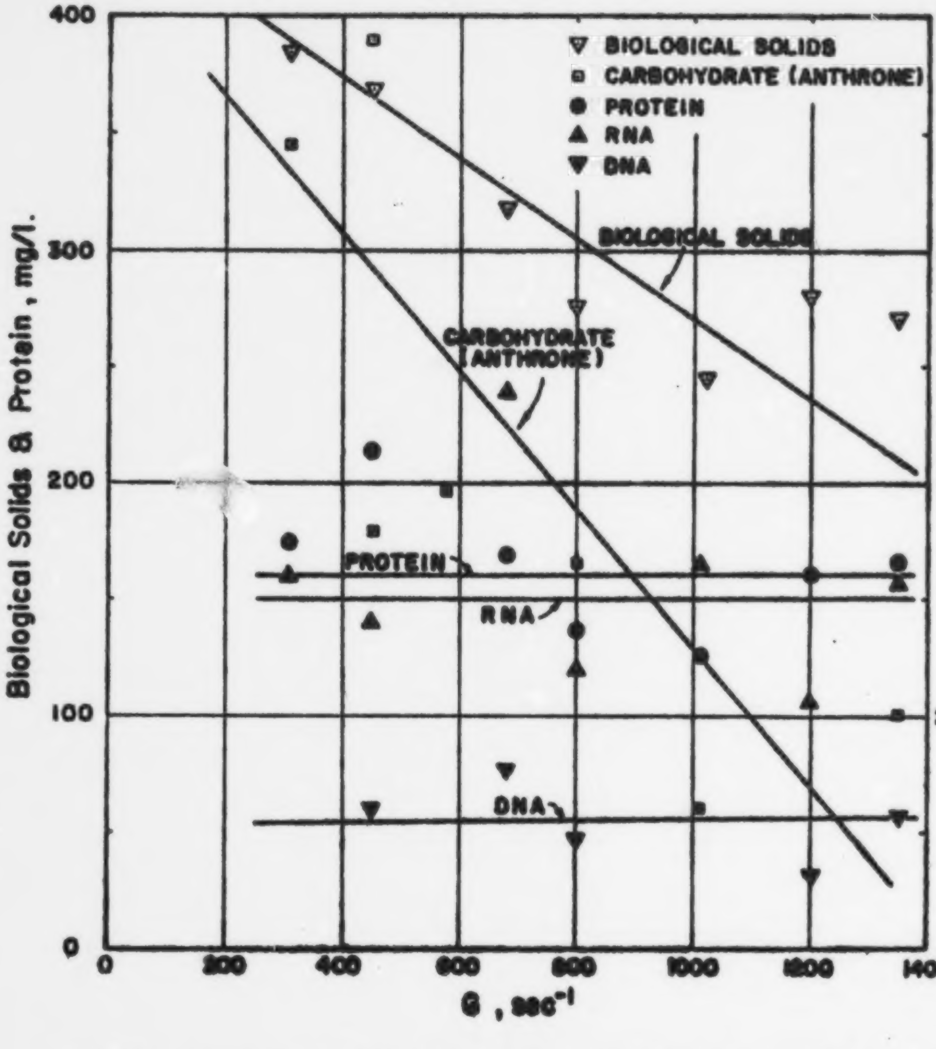

Fig. 8 Variation in Cell Composition with Changing Velocity Gradient.

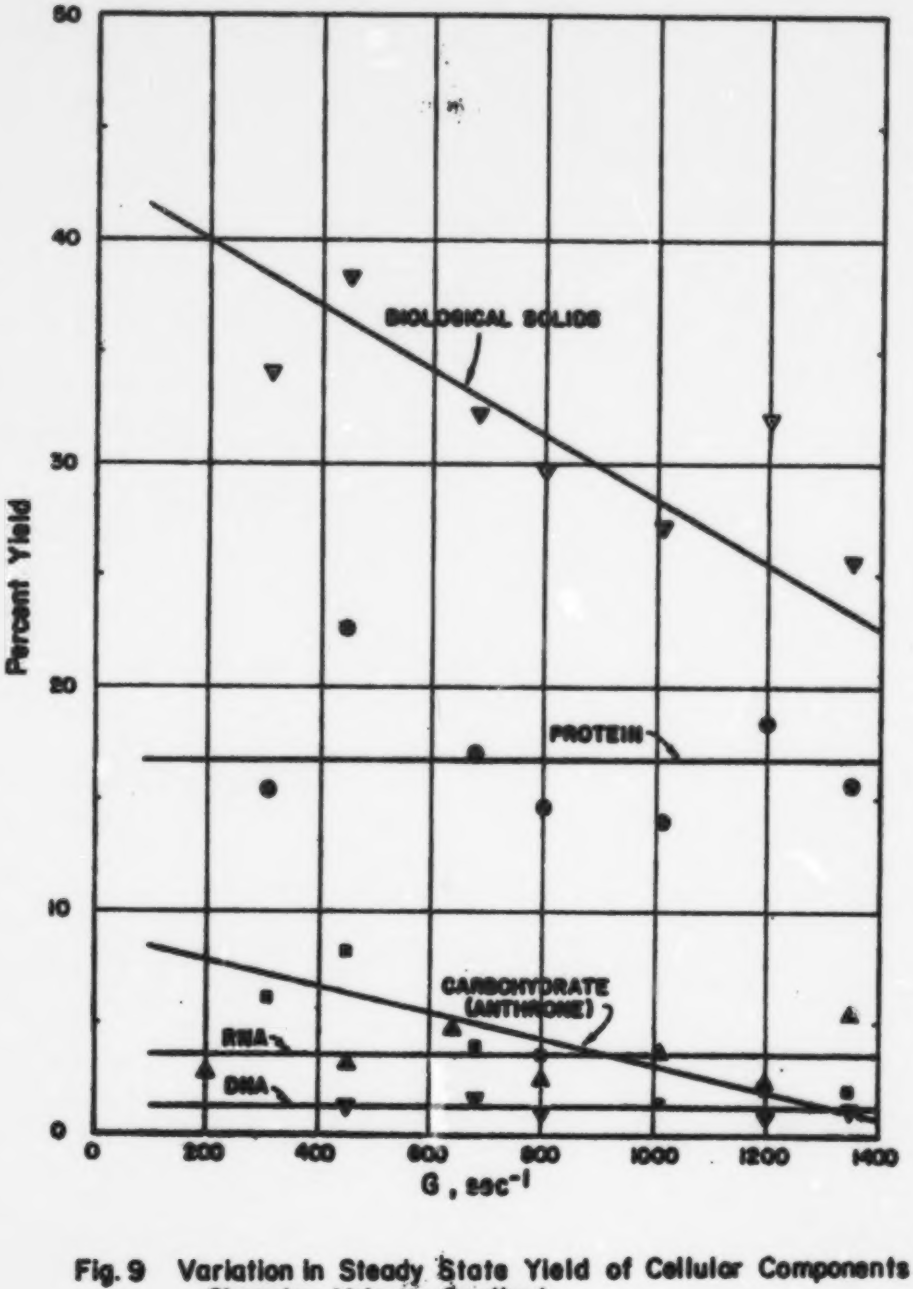

Fig. 9 Variation in Steady State Yield of Cellular Components with Changing Velocity Gradient .

of the calculated yields of biological solids, cellular protein, carbohydrates, RNA, and DNA. From this figure it is apparent that a significant reduction in the biological solids yield was realized as the velocity gradient was increased. A major portion of this reduction was due to reduced production of cellular carbohydrates. It is of further interest to note that no significant changes in the so-called "vital" cellular material, that is, protein, RNA, or DNA, occurred.

The composition of the sludge obtained during operation at the various velocity gradients, expressed in percentage of the biological solids, is shown in Figure 10. Here it may be seen that the protein content of the sludge tended to increase as the velocity gradient was increased, while the carbohydrate content decreased.

The steady state oxygen uptake from each experiment is plotted versus the velocity gradient in Figure 11. These data indicate that an increase in oxygen uptake occurred as the velocity gradient was increased.

A materials balance was made on all of the runs to obtain an indication of the validity of the relationship between the solids yield and the velocity gradient. These data, expressed in terms of equivalent glucose, are shown in Table II. An average of 91.6 per cent (range 77 to 101 per cent) of the glucose consumed was accounted for by the sum of respiration and synthesis. This value compares favorably with materials balances reported for "batch" experiments where Warburg respirometry was used to deter-

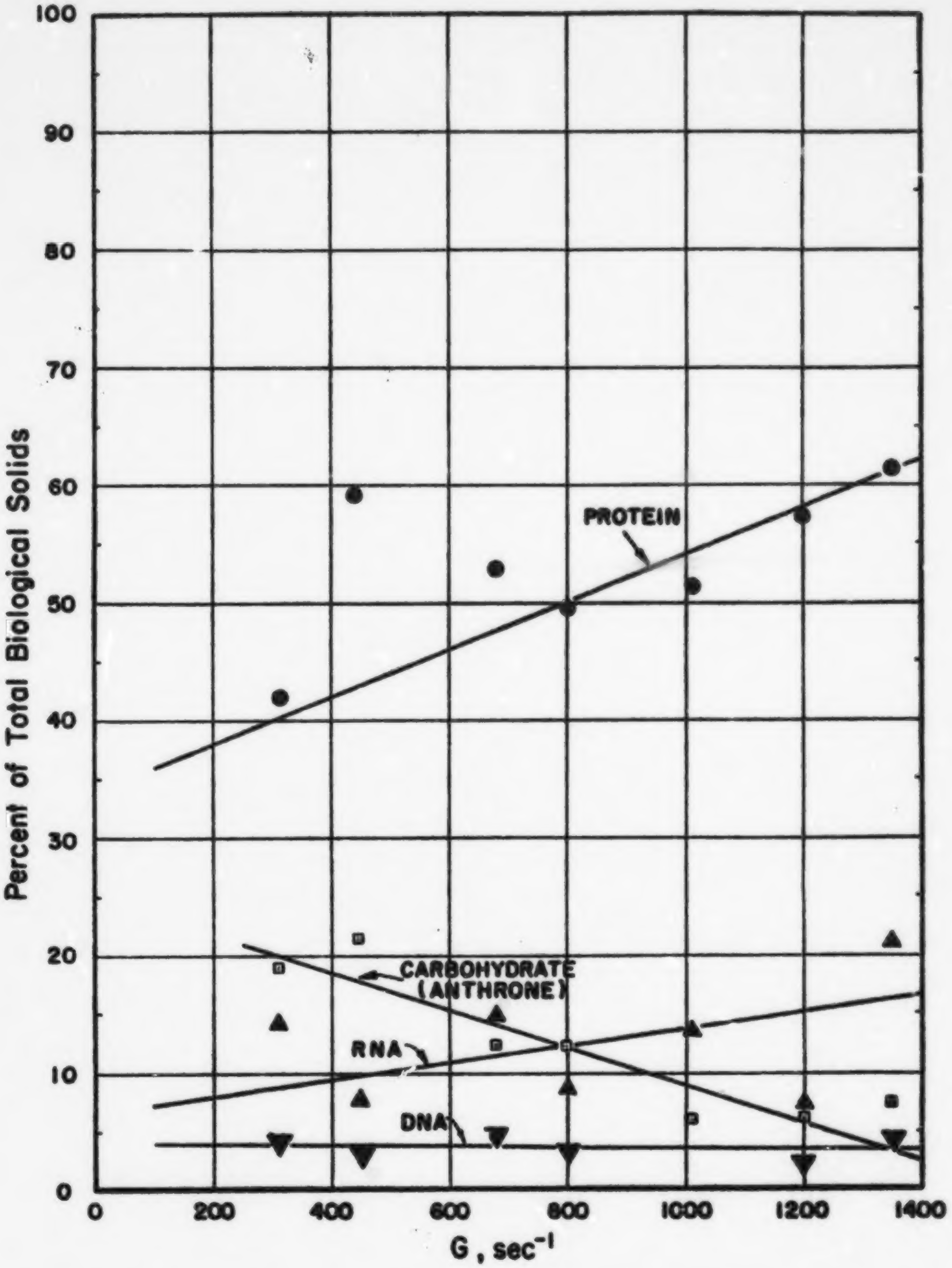

Fig. 10 Variation in Steady State Sludge Composition with Changing Velocity Gradient.

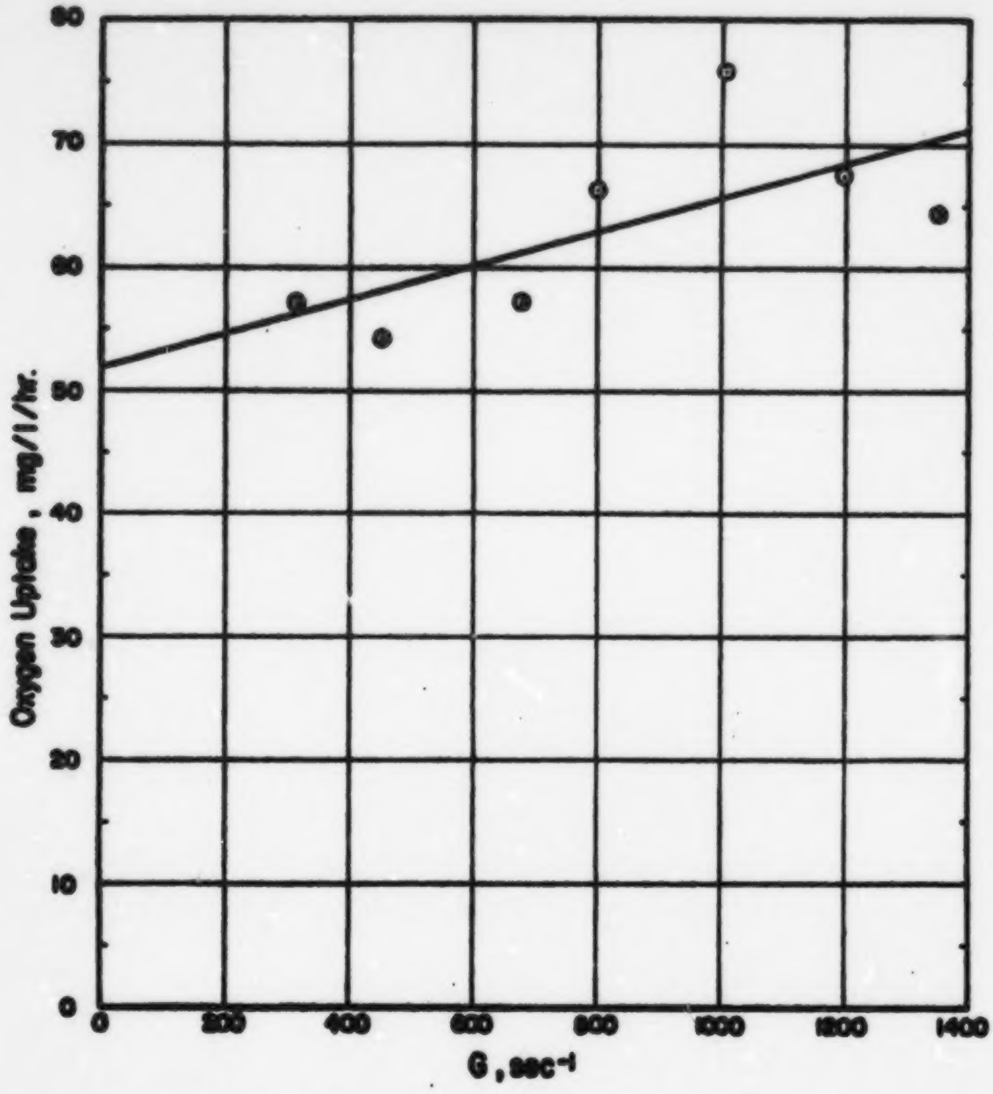

Fig. 11 Variation in Steady State Oxygen Uptake with Changing Velocity Gradient.

Table II

Materials Balance and Substrate Removal for Steady State Systems

Velocity Gradient (sec^{-1})	Flow Rate ① (l/hr)	COD Consumed ② as Glucose (mg/hr)	Oxygen Uptake as Glucose (mg/hr)	Average Solids ② Growth (mg/hr)	Total Glucose Accounted for (mg/hr)	Glucose Accounted for (%)	Carbohydrate Removed (Anthrone) (%)	COD Removed (%)
310	0.490	432	228	184	412	95	98	93
450	0.483	426	209	177	386	91	99	96
680	0.476	423	218	153	371	88	98	95
800	0.473	413	250	129	379	92	99	97
1010	0.467	388	281	112	393	101	99	92
1200	0.462	394	250	134	384	97	99	98
1350	0.459	470	237	127	364	77	99	94

① - Flow Rate adjusted for reactor volume change at various velocity gradients.

② - Rate of Consumption or Growth = [Influent Concentration (mg/l) - Effluent Concentration (mg/l)] x [Flow Rate (l/hr)] mine oxygen uptake (19). Also included in Table II are the percentage removals of substrate calculated as both total COD and glucose (anthrone). These values show that for the eight-hour dentention time employed in these studies, no significant change in substrate removal occurred as the energy input was varied.

In order to determine whether changes in predominance comparable to those observed by Zahradka (7) would occur in the system under study here, microscopic examination of the sludge was conducted during operation at each velocity gradient. These observations clearly indicated that the number of filamentous organisms present decreased as the amount of mechanical energy was increased. At 310 ft/sec/ft the majority of the cells present appeared to be of the filamentous type. Increasing the velocity gradient resulted in these organisms becoming less frequent until at 1010 ft/sec/ft they appeared to be totally absent.

DISCUSSION

The considerable reduction in the solids yield observed with increasing velocity gradient may prove to be of considerable importance in the design and operation of activated sludge systems. Although meaningful economic comparisons cannot be made with the limited data currently available, the use of increased mixing energy to achieve a reduction in sludge yield appears to be a highly interesting area for continuing investigation. This prospect is particularly attractive since, at the detention time employed, the reduction was accomplished with no deterioration in effluent quality. The fact that the same COD removal was maintained at lower solids levels as the velocity gradient was raised indicates that increased mixing resulted in the development of a sludge having a higher capacity for substrate removal per unit of mass. This contention is also supported by the fact that the amount of protein and nucleic acids produced remained essentially constant with varying agitation even though the solids decreased. As shown by Figure 10, this resulted in a sludge having a higher protein content at the higher velocity gradients. In addition to the reduction in sludge production, the predominance changes observed in these studies and those of Zahradka (7) indicate that control of the velocity gradient of activated sludge may provide control over the development of filamentous organisms.

On the basis of the literature cited earlier and the work presented here, five mechanisms can be advanced to explain the increase in oxygen uptake and the decrease in solids yield observed with increasing velocity gradient. These are:

1. Changes in predominance.

2. Increased frequency of contact between cells and substrate.

3. Production of smaller floc particles with resultant improved penetration of substrate and oxygen.

4. Increases in the rate of oxygen transfer across the cell-liquid interface.

5. Maintenance of a higher dissolved oxygen concentration with increasing turbulence.

At this stage, no actual proof of any of the above mechanisms is available. It should be pointed out, however, that changes in predominance, while they did occur in these experiments, cannot explain the increased oxygen uptake and accelerated production of gluconic acid by P. ovalis cited earlier (1)(2). In addition, as noted by Zahradka (7), predominance changes away from filamentous organisms would be expected to result in an improvement of sludge settleability but not necessarily in the improvement in purification rate observed by that author.

Improved frequency of contact and its related mechanism, the production of smaller floc particles, both may be used to explain the increased purification ability observed by Zahradka (7) and the increased oxygen uptake seen by others (1)(2). It is not understood, however, how the presence of physically smaller aggregations of cells or improved frequency of contact alone could account for the reductions in yield and in sludge carbohydrates observed in these studies.

The fourth mechanism, that of increased oxygen transfer into the cell at high mechanical energy inputs, depends on the surmise that a resistance to oxygen transfer exists at the cell-liquid interface. This opposition to oxygen uptake has been proposed by Swilley, Bryant and Busch (12) to be analogous to gas-liquid transfer and thus to be a function of the dissolved oxygen concentration of the medium and the transfer coefficient of the cells involved. Tsao and Kempe (2), on the other hand, obtained a dimensionally homogeneous model for oxygen uptake that was independent of the oxygen tension of the bulk medium. This model predicted that the oxygen uptake of a given culture should be a function of the air flow rate, the rate of agitation, the fluid properties of the medium, and the physical dimensions of the impeller. It should be noted that this model can be simplified considerably, while preserving dimensional homogenity by substitution of the velocity gradient, G, for the gas flow rate, the impeller velocity and the dimensions of the impeller. The verification of the concept of a critical oxygen concentration in bacterial systems by many workers makes the model of Tsao and Kempe particularly attractive as an explanation for the variation in oxygen uptake as a function of the velocity gradient. Briefly, this concept states that a value of dissolved oxygen exists in an aerobic culture above which no change in oxygen uptake will occur as the dissolved oxygen is further increased. Below the critical value, the rate of oxygen uptake is a function of the rate of gas-liquid transfer of oxygen into the medium. Thus, it may be theorized that the critical dissolved oxygen concentration is the oxygen tension at which the oxygen uptake of the cells ceases to be governed by the mass transfer of oxygen from the gas to the liquid phase and becomes dependent instead on some other limiting factor such as the cell-liquid interfacial resistance. Thus, the model presented by Tsao and Kempe (2) would apply only to systems where the dissolved oxygen concentration remained above the critical level. Within these limits, this model, as modified herein, predicts that the oxygen uptake of a steady state system should vary as a simple function of the velocity gradient. The data presented in Figure 11 show that this is apparently the case, since the oxygen uptake showed a definite tendency to increase with the velocity gradient.

The reduction in the carbohydrate yield as the mixing energy was increased may also be explained if consideration is given to the properties of carbon-limited, steady state systems. In systems of this type the growth rate of the cells is controlled entirely by the dilution rate. Thus, at a given detention time, the velocity gradient should have no effect on the growth rate of the cells. This being the case, it would be expected that the rate of synthesis and hence, the steady state levels of protein, RNA, and DNA would be maintained at a constant level as long as the dilution rate was not altered. The rate of synthesis of cellular glycogen, however, need not be maintained, since the function of this constituent is merely to provide energy storage. Thus, any increase in the rate of oxygen uptake might be expected to result in a decrease in the rate of glycogen synthesis and therefore, in the overall carbohydrate content of the cells, since the total amount of substrate available remained the same.

On the basis of the data presented here, the variation in the rate of oxygen supply which accompanied the changes in velocity gradient (Figure 4) cannot be eliminated as a factor in the variation in cell yield and composition. It should be noted, however, that the available data on the effect of oxygen supply rate show that at very low rates of oxygen supply the mass and viable count yield increase linearly with the rate of addition of oxygen. Phillips and Johnson (20) were able to demonstrate, however, that this effect was caused by oxygen deficiency and that no correlation existed between oxygen supply rate and yield if the oxygen tension was maintained above 0.01 - 0.02 atmospheres (0.4 - 0.8 mg/l) depending on the organism used. These data make it appear that the response of cell yield to oxygen tension is similar to the response of oxygen uptake; that is, the cell yield is not affected by oxygen tension if the culture is maintained above a critical DO. On this basis, it appears unlikely that the variation in solids yield with velocity gradient (Figure 9) can be attributed to the increased oxygen tensions which occurred at the high agitation rates. In addition, comparison of Figures 7 and 9 indicates that the order of the relationship between the DO in the reactor and the velocity gradient was apparently different than that of the relationship between the cell yield and the velocity gradient. Although considerable scatter existed in the data, probably caused by population variations in the heterogeneous system, the reduction in cell yield observed with increasing velocity gradient was roughly linear, whereas the increase in dissolved oxygen with increasing velocity gradient appeared to be more nearly first order.

The increase in the protein content of the sludge with increased mixing (Figure 10) is not believed to be caused by increases in the viable population, since this should have resulted in higher DNA levels. Figures 8 and 9 show that no such increase in either the RNA or DNA occurred. Rather, the higher protein content is more likely caused by the fact that the reduction in solids yield was accomplished primarily at the expense of cellular carbohydrate with no significant change occurring in the protein yield (Figure 9). This would result in a higher protein content merely by decreased dilution from other cell constituents.

The extremely high levels of substrate removal observed in all of the experiments shown here make it difficult to assess the effect of velocity gradient on substrate utilization. However, it is apparent that the unit ability of the sludge to consume substrate (at the dilution rate employed) was enhanced as the rate of agitation was increased as shown by the maintenance of the high COD removal at lower solids concentrations.

CONCLUSIONS

Operation of a steady state bacterial system at a constant dilution rate with varying agitation rates has shown that solids yield and oxygen uptake both vary with the amount of agitation provided. The decrease in cell yield with increasing turbulence appears to occur primarily at the expense of cellular carbohydrate. This resulted in the production of a sludge having a higher protein content at the higher agitation rates.

Five mechanisms which may be used to explain the effects observed are presented and discussed. Although none of these mechanisms can be eliminated from consideration on the basis of the data currently available, it is felt that the increased oxygen uptake with increased turbulence most likely resulted from reductions in the cell-liquid interfacial resistance to oxygen transfer as the amount of mixing energy was increased. The decrease in biological solids yield which occurred primarily at the expense of cellular carbohydrate is believed to have resulted from an increased ratio of respiration to synthesis, i.e., a reduction in synthesis of stored material rather than from a reduction in the viable population. Additional work is currently underway which should provide greater insight into the observed variations.

ACKNOWLEDGEMENT

This work was supported in part by a training grant (2T1-WP-19-06) and in part by a research grant (WP00325) from the Federal Water Pollution Control Administration, Department of the Interior.

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PAPER 25

Rickard, M. D., and Gaudy, A. F. Jr., "Effect of Oxygen Tension on O_2 Uptake and Sludge Yield in Completely Mixed Heterogeneous Populations." Presented at the 23rd Industrial Wastes Conference, Purdue University, May, 1968.

EFFECT OF OXYGEN TENSION ON O_2 UPTAKE AND SLUDGE YIELD IN COMPLETELY MIXED HETEROGENEOUS POPULATIONS

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INTRODUCTION

The oxygen requirements of aerobic bacterial systems have been a subject of continuing research interest in the field of microbiology, and an excellent understanding of the role of oxygen tension in the growth and biochemical behavior of pure culture systems has been obtained. In general, it may be stated that the metabolism of a given microorganism has been found to be independent of the oxygen tension if the dissolved oxygen concentration is maintained above a minimum value, 0.2 - 0.8 mg/l (1)(2), termed the critical D.O. Although the critical D.O. originally referred only to that oxygen tension at which respiration rate became independent of oxygen tension, additional studies (1)(3) have demonstrated that viable count and cell mass yield also are not affected by changes in dissolved oxygen above this level.

Presented at the 23rd Industrial Waste Conference, Purdue University, Lafayette, Indiana, May, 1968.

Unfortunately, in the field of biological waste treatment the relationship between dissolved oxygen concentration and cell growth has not been so well defined because these microbial systems employ much higher biological solids concentrations than are normally used in microbiological studies and because naturally occurring heterogeneous populations are subject to as yet unpredictable variations at the ecological level. Therefore, the application to biological waste treatment systems of relationships derived from studies using pure cultures at relatively low solids concentrations must be viewed with caution. In addition, short term "batch" investigations, even when conducted on heterogeneous populations, may not allow the full expression of selective pressures and hence may not provide a full assessment of all factors involved.

The investigation reported in this paper was conducted in an attempt to determine the effect of dissolved oxygen concentration on the behavior of a natural population under steady-state, continuous flow conditions. It was hoped that an investigation of this type would provide more complete understanding of the response of waste treatment systems to oxygen tension at both the biochemical and ecological levels.

A number of investigators (3)(4)(5) have demonstrated the existence of a critical D.O. for activated sludge in terms of both respiration rate and substrate removal. However, Heukelekian (6) observed that the viable count developed in a BOD bottle varied over wide limits as the initial D.O. was changed. He attributed this variation to the development of different populations at the various D.O. levels. In addition, Swilley, Brant, and Busch (7) hypothesized that in some systems a stagnation layer might exist around the bacterial cell which would cause oxygen transfer into the cell, and hence respiration rate, to be governed by mass transport. Pasveer (8)(9)(10)(11) observed that the permissible loading of a brush aeration activated sludge unit was a function of the amount of agitation provided. He attributed this improvement to the maintenance of smaller floc particles in the aeration basin at high agitation rates, which led to improvement in the transport of oxygen into the cells. Zahradka (12) observed that increased agitation in an activated sludge pilot plant resulted in increased efficiency of substrate removal. This was also attributed to the maintenance of smaller floc particles.

In addition to the above work, a number of investigations (13)(14)(15)(16) have demonstrated the existence of a definite relationship between oxygen uptake and the amount of mixing energy provided to the system. Two of these investigations (14)(16) considered the possibility that these effects might be attributed in part to variations in dissolved oxygen, since the oxygen tension in these studies increased with increasing agitation.

THEORETICAL CONSIDERATIONS

In his thorough study of the effect of turbulence on the behavior of activated sludge systems, Pasveer (8) (9) (10) (11) found that as the amount of agitation furnished to the aeration unit was increased, the permissible BOD loading increased. He attributed this to the fact that high fluid shear reduced the size of the floc particles contained in the aeration chamber. This, in turn, allowed better penetration of oxygen into the floc matrix. Pasveer was able to show that if oxygen transfer into the floc was rate-limiting and if the entire floc was aerobic, then the oxygen uptake rate per floc, Q_1, should be given by:

$$Q_1 = \frac{4\pi r^3}{3} \alpha \frac{4T_p + T_m}{5} {}^{*} \qquad 1$$

where r is the radius of the floc particle, α is the amount of oxygen taken up by one cc of floc volume at an oxygen concentration of 1.0 gm/cc, T_p is the oxygen tension at the center of the floc particle, and T_m is the oxygen concentration at the water-floc interface. Pasveer further stated that the factors T_m and T_p could be evaluated from the relationships:

$$T_m = T_p \frac{15k - 4\alpha r^2}{15k + \alpha r^2} \qquad 2$$

and

$$T_p = \frac{T}{1 + \frac{2.5\,\alpha r}{15k + \alpha r^2}\sqrt{\pi k t'}} \qquad 3$$

---

[*] T, T_m and T_p are used for oxygen concentration for conformity with following formulations.

where k is the diffusion constant of oxygen in water, T is the oxygen tension of the bulk medium, and t' is the hypothetical time of subsistence of the static water-floc interfacial surface. Similar relationships were developed for the case where only a portion of the floc receives oxygen, but, as the kinetics of the two cases are identical, only the totally aerobic floc will be considered here.

Examination of equations 1, 2, and 3 shows that these relationships may be combined into a single expression relating oxygen uptake to the size of the floc particle, the number of floc particles/unit volume and the oxygen tension of the bulk medium. However, in a steady-state system operating at a constant detention time and agitation rate the number of flocs/unit volume, t' and r may be expected to be constant so that the oxygen uptake may be represented as the product of a single constant K_1:

$$U = K_1 T \qquad 4$$

where U is the rate of oxygen uptake/unit time. Therefore, if the size of the floc particle limits the oxygen uptake rate of a bacterial system, the rate of oxygen uptake at steady-state should vary directly with the oxygen tension.

Swilley, Brant, and Busch (7) in their theoretical considerations concerning the existence of a stagnation layer around the bacterial cell show that, if such a layer existed, the oxygen uptake of a bacterial system should be governed by mass transport. Since the rate of oxygen uptake would in this case be equivalent to the rate at which oxygen was being transferred into the cell, it would be expected that oxygen uptake rate would be proportional to the concentration of oxygen maintained in the medium:

$$U = K_2T \qquad 5$$

where K_2 is a mass transfer coefficient. This relationship indicates a zero order relationship between oxygen tension and oxygen uptake similar to equation 4.

Rincke (17) in his discussion of the work of Zahradka, postulated that improved mixing might account for the variations in oxygen uptake observed as agitation is increased. This concept may be tested using the relationship given by Fair and Geyer (18) to relate the number of contacts occurring between particles in suspension to velocity gradient. According to these authors, the number of contacts occurring between two classes of particles having concentrations of n' and n" in terms of numbers/unit volume is:

$$N = \frac{n'n''(d' + d'')^3}{6} G \qquad 6$$

where N is the number of contacts per unit time, d' and d" are the respective particle diameters, and G is the velocity gradient.

Considering the case of contact between the cells and the oxygen molecules present and assuming that an oxygen molecule has a diameter of zero:

$$N = \frac{nd^3X_n}{6} G \qquad 7$$

where n is the number of oxygen molecules per unit volume, d is the mean cell diameter, and X_n is the viable microbial count. Therefore

$$U = f_o k_3 N = f_o k_3 \frac{nd^3 X_n}{6} G \qquad 8$$

where f_o is the fraction of collisions which result in uptake and k_3 is the unit mass of an oxygen molecule. However

$$n = T/k_3 \qquad 9$$

so that equation 8 reduces to

$$U = \frac{Td^3 X_n}{6} f_o G \qquad 10$$

At steady-state and constant G, X_n, f_o and d may be expected to be constant so that

$$U = K_4 T \qquad 11$$

In summary, the above discussion indicates that if O_2 uptake of an activated sludge system at steady-state is controlled by floc diameter, by mass transport into the bacterial cell or by mixing, then the oxygen uptake rate should be a function of the oxygen tension. In other words, the above formulations do not predict independence of oxygen uptake and oxygen tension above the critical D.O. Thus, if oxygen uptake is found not to increase as a function of the dissolved oxygen concentration in steady-state systems containing a natural population, the above concepts can be eliminated from consideration as rate-controlling factors for the experimental conditions employed.

In addition, if the cell mass yield of such a system can also be shown to be independent of oxygen tension, it appears unlikely that the variations in sludge yield at various values of velocity gradient, G, previously observed by Rickard and Gaudy (16) can be attributed to ecological selection induced by changes in oxygen tension.

MATERIALS AND METHODS

Experimental Unit

A 5-liter Microferm laboratory fermenter (New Brunswick Scientific Co.) was used throughout these studies. Aeration of the medium was provided by a single air outlet in the bottom of the culture vessel through which filtered gas, consisting of a known mixture of oxygen and nitrogen, was passed. Although designed for batch operation, the unit was converted to continuous flow by addition of an influent feed line and an inverted standpipe, positive suction effluent line. Sigmamotor AL4E pumps were used on both the influent and effluent lines. A schematic diagram of the unit as modified is shown in Figure 1. The reactor is identical to the one used in previous studies (16).

Medium Composition

The medium used in these studies consisted of a glucose-mineral salts solution having the following composition: glucose, 1000 mg/l; $(NH_4)_2SO_4$, 500 mg/l; $MgSO_4 \cdot 7H_2O$, 200 mg/l; $MnSO_4 \cdot H_2O$, 20 mg/l; $CaCl_2$, 15 mg/l; $FeCl_3 \cdot 6H_2O$, 1.0 mg/l; tap water, 100 ml/l; 1.0 M phosphate buffer, 15 ml/l.

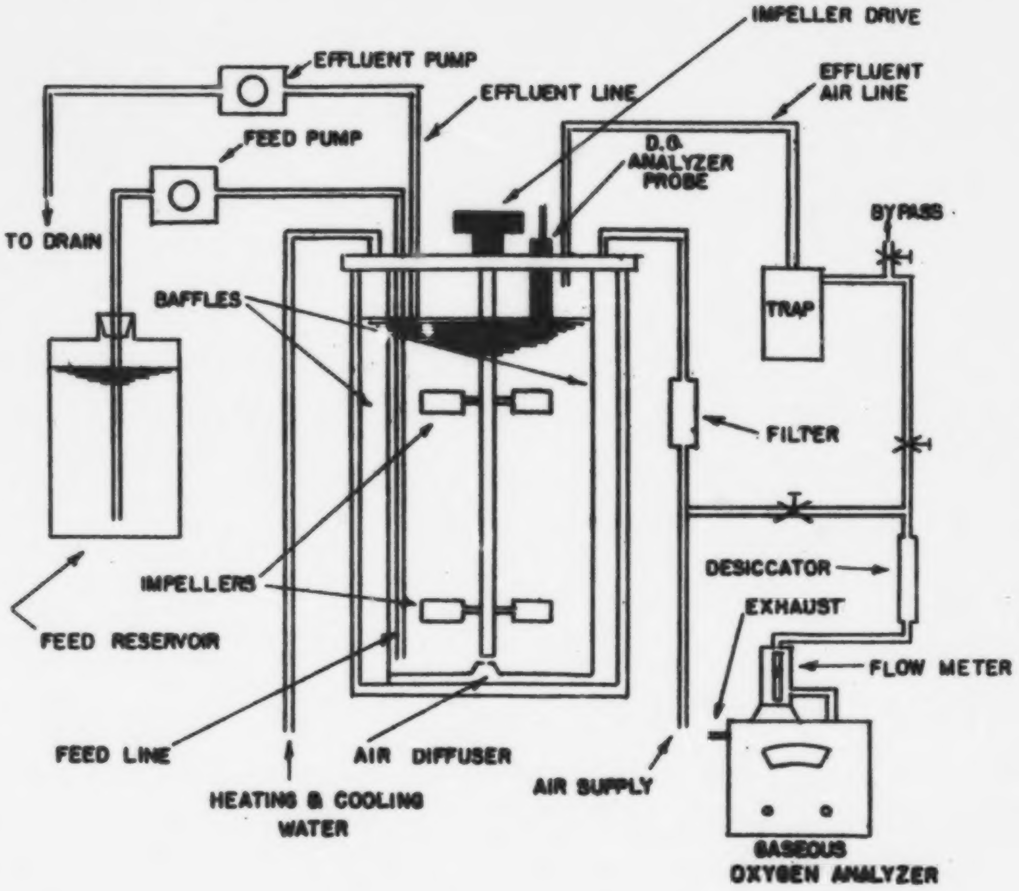

Figure 1. Schematic Diagram of Experimental Unit.

Experimental Protocol

The experimental unit was started by filling the reactor with the standard glucose medium and adding 100 ml of primary effluent from the Stillwater, Oklahoma, municipal waste treatment plant. Operation was then conducted in a "batch" manner for twenty-four hours at 30°C to allow the development of a vigorous microbial population. Pumping was then begun and the unit was operated for seventy-two hours before an experiment was started. During the last twenty-four hours before sampling was begun and prior to the start of each subsequent run, the unit was checked for steady-state conditions by determining the optical density (Bausch and Lomb Spectronic 20, 1 cm light path, 540mμ) of a number of samples over the twenty-four hour period. In all cases the unit achieved steady-state with respect to optical density before the start of each experiment.

Four runs were conducted using three different oxygen-nitrogen gas mixtures, a detention time of eight hours, a temperature of 30°C, a gas flow rate of 1.0 l/min, and constant velocity gradient, G, of 1000 sec^{-1}. Gas mixtures containing 5% oxygen-95% nitrogen, 10% oxygen-90% nitrogen, and 21% oxygen-79% nitrogen were used to span dissolved oxygen concentrations which might be encountered in activated sludge units. No less than six samples were taken for each parameter during operation at a given D.O. The sampling period extended over three detention times. The average value of each parameter was then taken to represent the steady-state value.

Analytical Methods

The biological solids level in the reactor was determined by the membrane filter technique. Substrate utilization was determined by analyzing the membrane filtrate for both COD (19) and glucose (by anthrone)(20). The influent COD and glucose concentrations were also determined so that the yield of the various cellular parameters could be calculated. Samples for cell composition analysis were taken by the method outlined by Gaudy (20). This resulted in a washed cell suspension having ten times the solids concentration of the culture sampled. One ml of this suspension was subjected to hot perchloric acid extraction according to the method of Burton (21) for subsequent nucleic acid analysis. The remainder (4 ml) was frozen and stored for carbohydrate and protein determination.

RNA analyses were performed on the perchloric acid extract according to the orcinol method reported by Ceriotti (22), except that extraction of the final colored product with isoamyl alcohol to achieve concentration of the color was eliminated as it was shown to be unnecessary with the concentrations found in the samples obtained.

DNA was determined on the perchloric acid extract by the diphenylamine reaction as given by Burton (21).

Carbohydrates and protein contents of the washed cell suspension were determined by the anthrone and biuret methods, respectively (20).

The dissolved oxygen content of the medium was determined continuously during steady-state operation by a galvanic cell oxygen analyzer (Precision Scientific Co.) connected to a 10 mv recorder (E. H. Sargent Co.).

Oxygen uptake during steady-state growth was determined by measuring the difference in oxygen content between the influent and effluent gas streams, in accordance with the manufacturers' recommendations (23) using a Beckman Model E2 gaseous oxygen analyzer. This instrument allowed the determination of the oxygen content of the gas stream with a precision of 0.01%. The difference between the oxygen content of the influent and effluent gas streams was then converted to milligrams of oxygen consumed per hour, and the values divided by the volume of the medium in the fermenter (3.81 liters at the conditions employed) to obtain the rate of oxygen uptake in mg/l/hr.

RESULTS

The gas mixtures employed provided near steady state dissolved oxygen levels of 1.4, 3.6, and 7.1 mg/l. In order to illustrate the nature and magnitude of the variation which occurred in all of the parameters during steady state operation, the results of an experiment performed at a dissolved oxygen concentration of 7.1 mg/l are shown in Figures 2 and 3. As previously noted, the steady-state level of each parameter was taken as the average of at least six independent observations of each parameter.

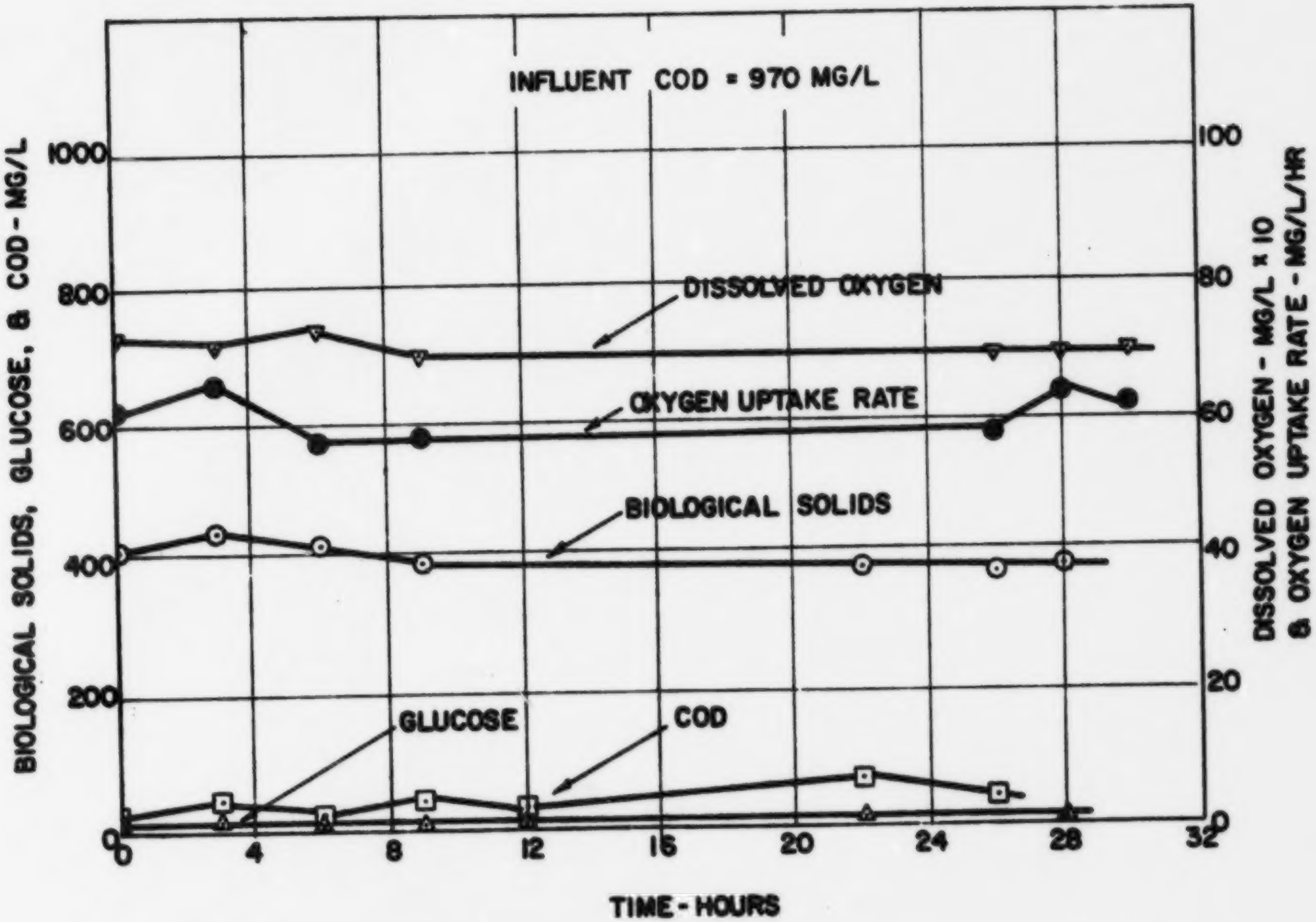

Figure 2. Steady-state Parameters during Operation with Aerating Gas Containing 21% Oxygen at a Dilution Rate of 0.125 hr^{-1}.

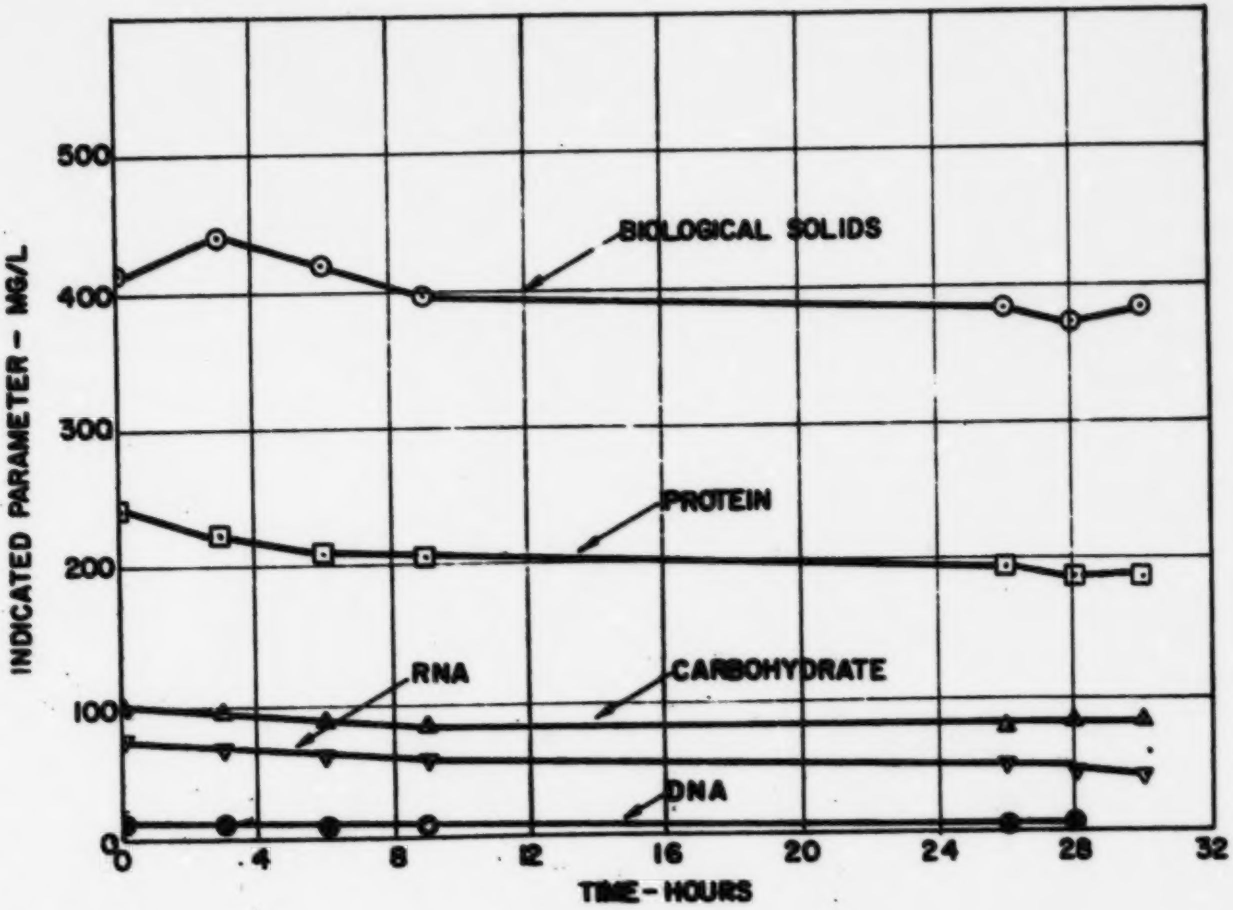

Figure 3. Concentrations of Cellular Components during Operation with Aerating Gas Containing 21% Oxygen at a Dilution Rate of 0.125 hr^{-1}.

The average results of the steady state determinations of biological solids yield and oxygen uptake rate at the various D.O. concentrations are shown in Figure 4. From this figure it is seen that no significant variation in either parameter occurred as the dissolved oxygen concentration was varied. This is in sharp contrast to the results obtained by Rickard and Gaudy (16) when the agitation rate was changed.

The composition of the sludge produced at the various oxygen tensions studied is shown in Figure 5. Here it may be seen that while neither the protein nor RNA content of the cells varied appreciably, a significant reduction occurred in the carbohydrate content of the cells as the oxygen tension increased. The DNA content of the cells increased slightly with the dissolved oxygen concentration.

The substrate removal attained in the unit at the various dissolved oxygen levels is shown in Figure 6. There was a slight reduction in the concentration of both COD and glucose in the effluent.

DISCUSSION

Swilley, Brant, and Busch (7) theorized that a stagnation layer might exist around the bacterial cell, which could result in the rate of oxygen uptake being a function of the amount of dissoved oxygen present. Heukelekian (6) found that the dissolved oxygen level of a culture containing a heterogeneous population could affect the viable cell count. The variations he observed in the count were

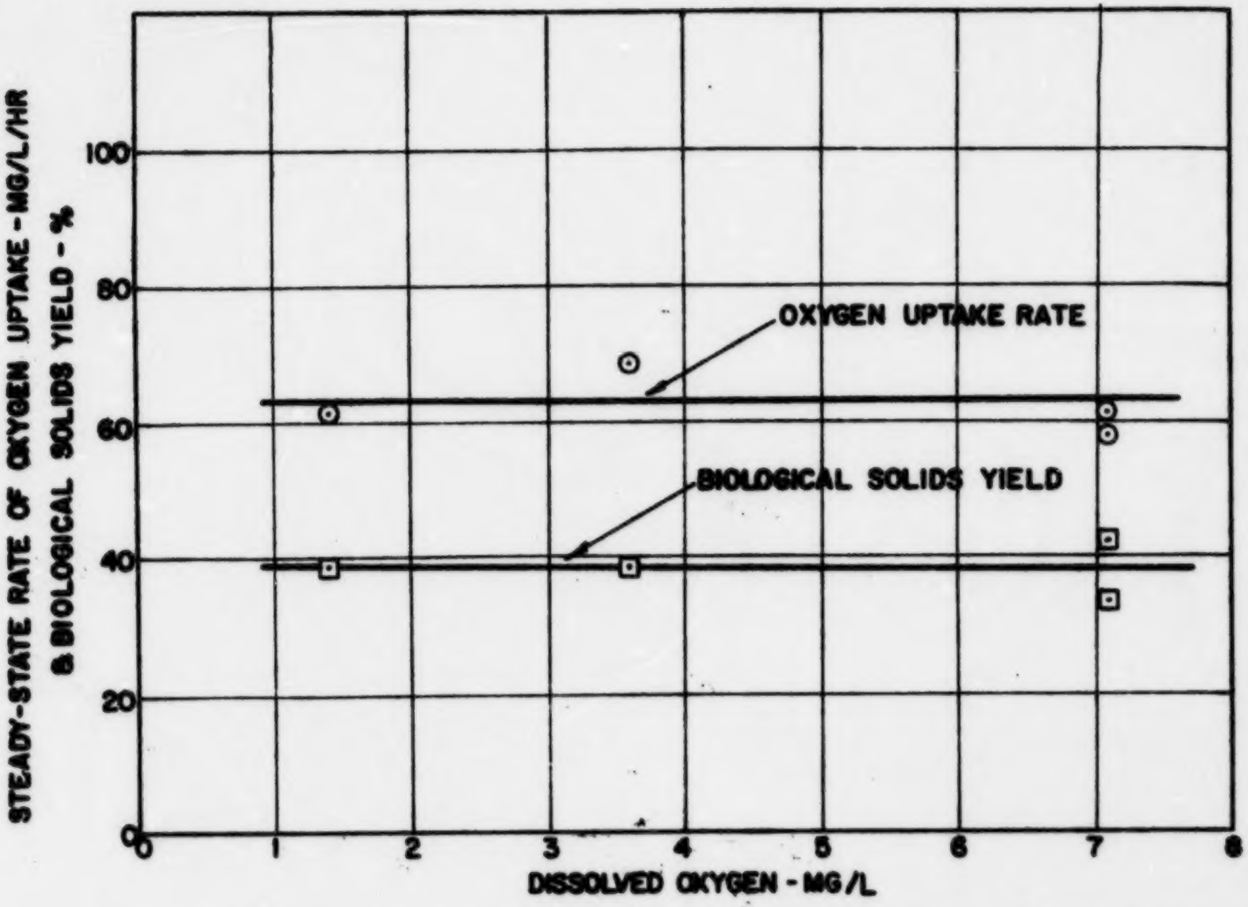

Figure 4. Steady-state Biological Solids Yield and Oxygen Uptake Rate at Various Oxygen Tensions.

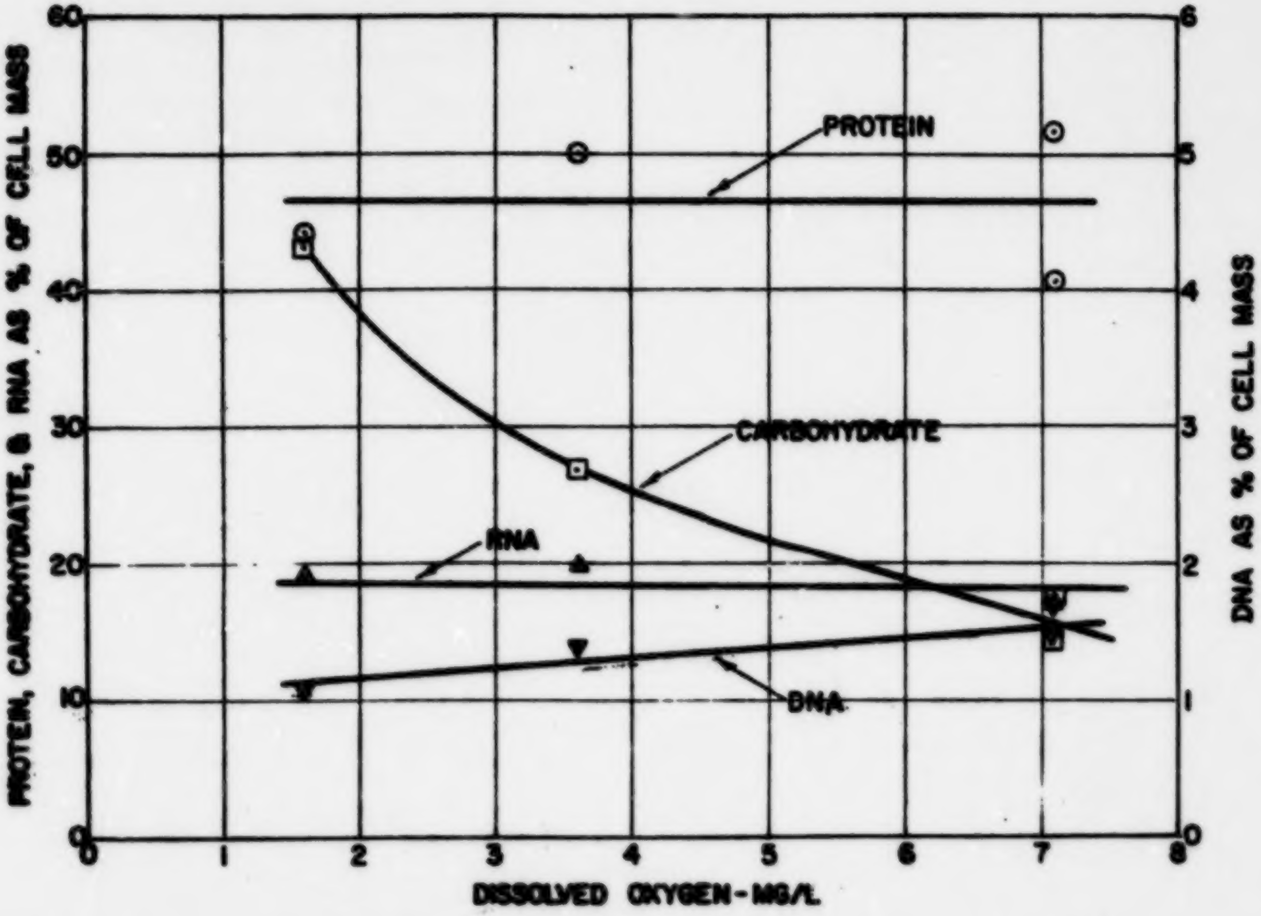

Figure 5. Composition of Sludge Obtained during Operation at Various Oxygen Tensions.

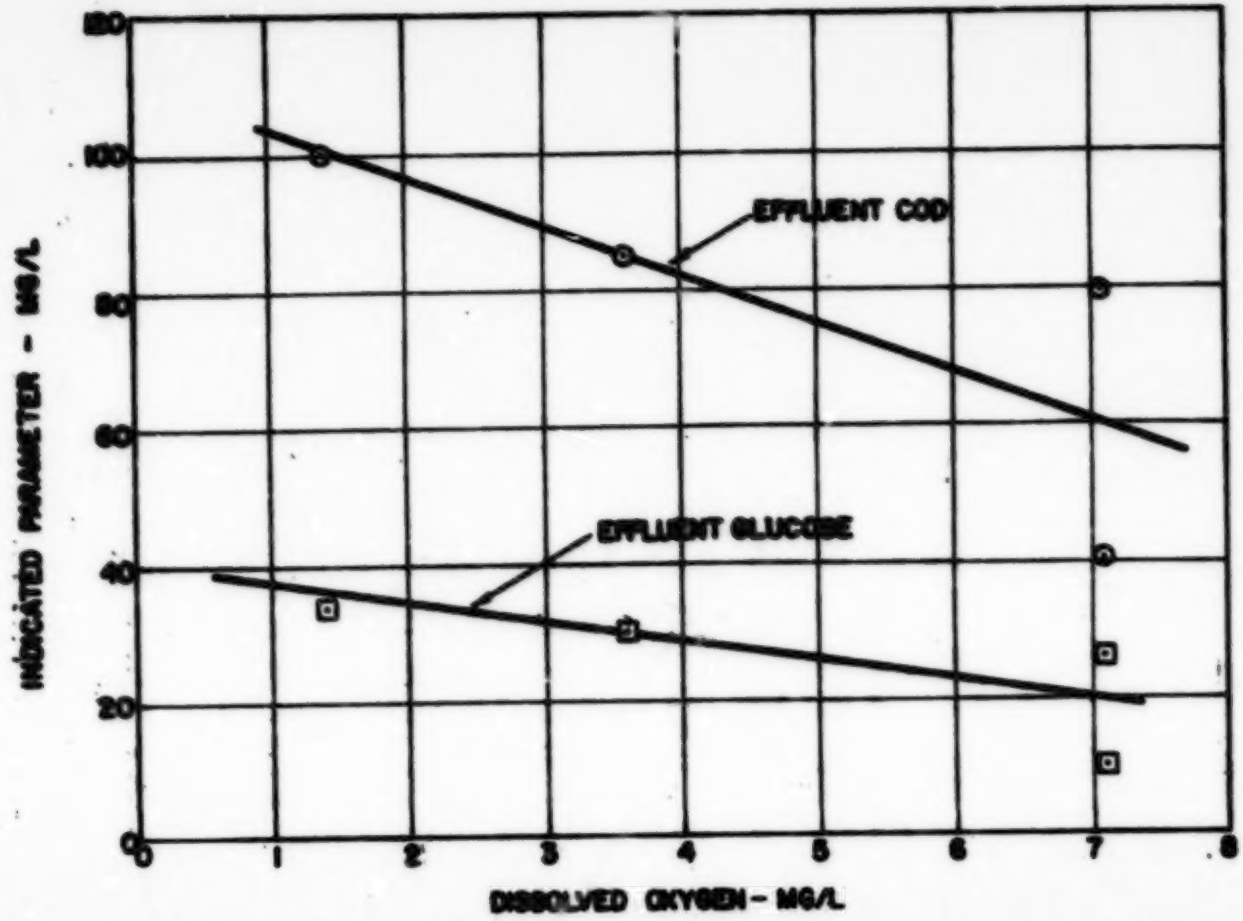

Figure 6. Effluent COD and Glucose during Operation at Various Oxygen Tensions.

attributed to changes in the predominating organisms. In addition, both the mechanisms proposed by Rincke (17) and by Pasveer (9)(10)(11)(12) to explain the effect of agitation on the behavior of activated sludge processes indicated that the oxygen uptake rate of a bacterial system should be a direct function of the dissolved oxygen in the medium. Since these concepts would not appear to be consistent with conclusions drawn from studies undertaken in the basic microbiological field, it was deemed desirable to determine whether the oxygen tension of a continuous flow steady state system at D.O. concentrations above those which could be considered to be critical concentration had an effect on the oxygen uptake rate or the biological solids yield. This was accomplished by operating a completely mixed, steady state reactor containing a heterogeneous population under conditions of constant growth rate and agitation and varying amount of oxygen contained in the aerating gas. These experiments have clearly demonstrated that dissolved oxygen had no effect on either the oxygen uptake rate or the biological solids yield. In light of these findings and from the considerable volume of information available attesting to the existence of a critical D.O. in various experimental systems, it may be concluded that while stagnation layers around the bacterial cell may or may not exist, they do not appear to be an important factor for governing O_2 uptake in well-agitated completely mixed systems. Also, it should be recalled that analysis of the concepts of Pasveer and of Rincke indicated that under constant mixing conditions (and assuming that under such conditions floc size would be relatively constant) oxygen uptake should increase for higher dissolved oxygen concentrations. However, such was not the case in the present study, in which a relatively high constant mixing gradient was employed, and dissolved oxygen concentration was maintained above the values which could be considered critical concentrations. In our previous work in which the oxygen tension was maintained above accepted values for critical D.O., it was found that increased agitation did cause an increase in oxygen uptake (16). Therefore, it seems apparent that while oxygen tension (above accepted critical values) exerts no effect upon oxygen utilization rate, agitation does exert an effect. Thus, it seems logical to conclude that the agitation in the system exerts an effect independent of oxygen tension. From the present work and the previous study (16) it is not possible to propose reasons for this independent effect, but it is possible to conclude that there is now experimental evidence showing that the oxygen tension (above critical D.O. values) does not affect O_2 uptake rate, and that agitation does exert an independent effect. Although there was no change in oxygen uptake rate or biological solids yield as oxygen tension was increased, there was a definite tendency for the amount of carbohydrate produced by the cells to decrease as the dissolved oxygen concentration was increased. It is conceivable that this variation in carbohydrate production arose as a direct effect of oxygen tension on the metabolic fate of the substrate. However, in the absence of variation in any of the other cellular parameters, and in light of the considerable body of evidence which indicates that the metabolism of bacteria is unaffected by changes in O_2 tension above a critical D.O., it is felt that such an explanation is unjustified. In any study employing heterogeneous populations, the possibility of changes in predominating species cannot be ignored as a causative factor for the change in cell composition which was noted; however, in the present studies there was no readily apparent change in predominating species. The fact that the protein and DNA content did not change tends to indicate that the change in cell composition was not due to a change in the total viable count. Viable cell counts were not made in these studies because of the semi-flocculated nature of the system. It is felt that additional data on carbohydrate production in bacterial systems under conditions of varying oxygen tension is necessary before a sound explanation for the decreased carbohydrate content can be advanced.

CONCLUSIONS

Operation of a completely mixed steady state reactor at constant agitation (velocity gradient) but varying oxygen tension has shown that oxygen uptake rate and biological solids yield remained relatively constant for the range of dissolved oxygen concentrations between 1.4 and 7.1 mg/l.

In addition, no effect was noted on the amount of RNA or protein contained in the cells. Also, as oxygen concentration was increased, there was a slight increase in DNA content of the cells, and a small improvement in both COD and glucose removal efficiency. There was a significant decrease in carbohydrate content of the cells. Concerning oxygen uptake rate, it may be concluded, based upon the present results and the work previously reported (16), that agitation of the system exerts an effect independent of oxygen tension at dissolved oxygen concentrations above accepted values for critical D.O. concentration.

ACKNOWLEDGEMENT

This work was supported in part under a training grant 5T1-WP-19 and in part under a research grant WP-00325, both from the Federal Water Pollution Control Administration, U. S. Department of the Interior.

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PAPER 26

Isaacs, W. P., and Gaudy, A. F. Jr., "A Method for Determining Constants of First Order Reactions from Experimental Data." Biotechnology and Bioengineering X, 69-82, 1968.

A METHOD FOR DETERMINING CONSTANTS OF FIRST ORDER REACTIONS FROM EXPERIMENTAL DATA

By

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A number of processes of interest in the bioengineering field proceed in accordance with first order kinetics. Two of the most important "reactions" are physical reaeration in receiving streams and exertion of biochemical oxygen demand. While the order of reaction of the latter process may be subject to much debate, it is usually assumed to proceed with kinetics typical of monomolecular reactions. The kinetic constants are usually determined from experimental data, and many methods have been proposed for the evaluation of the velocity constant, K_1, and the ultimate BOD, L_0 (1). However, many of these methods are either so tedious that their use is avoided, or so simplified that they provide only a gross approximation of the constants. It is the purpose of this paper to describe the use of a method for obtaining first order constants which was originally introduced by Davis (2) for use in adjusting data which did not show linearity when plotted on semilogarithmic paper. The authors have found that, in addition to its originally intended use, the method of Davis can be successfully employed to obtain accurate values of first order reaction constants.

The mathematics of first order reactions are well known, and only a brief review seems warranted. If the rate of a reaction or a process is found to be directly proportional to the amount of reactant present at any time, the rate may be expressed by the following equation:

$$\pm \frac{dC}{dt} = KC \qquad (1)$$

where C is the concentration of reacting material, t is time, $\pm \frac{dC}{dt}$ is the rate at which the concentration increases or decreases, and K is the velocity constant.

Upon integrating Equation (1), the following equation is obtained:

$$\pm \ln C = Kt + C_0 \qquad (2)$$

or

$$\pm \log C = \frac{Kt}{2.303} + \frac{C_0}{2.303} \qquad (3)$$

where C_0 is the concentration of reacting substance at zero time. Thus it can be seen from Equation (3) that a straight line is produced when the logarithm of the concentration is plotted against time. The slope of the line, log C versus time, is equal to the velocity constant $(k = \frac{K}{2.303})$.

If direct measurements of C can be made at periodic intervals during the course of reaction, the evaluation of the velocity constant K can be made by integrating Equation (1) between the limits, concentration C_1 at time t_1, and C_2 at a later time t_2; the resulting expression is:

$$\pm \int_{C_1}^{C_2} \frac{dC}{C} = K \int_{t_1}^{t_2} dt$$

Its evaluation yields

$$\pm \ln C_2 - (\pm \ln C_1) = K(t_2 - t_1).$$

Solving for the velocity constant:

$$K = \frac{1}{(t_2 - t_1)} \ln \frac{C_1}{C_2} \qquad \text{1st Order Decreasing} \qquad (4a)$$

and

$$K = \frac{1}{(t_2 - t_1)} \ln \frac{C_2}{C_1} \qquad \text{1st Order Increasing} \qquad (4b)$$

or

$$k = \frac{1}{(t_2 - t_1)} \log \frac{C_1}{C_2} \qquad \text{1st Order Decreasing} \qquad (5a)$$

and

$$k = \frac{1}{(t_2 - t_1)} \log \frac{C_2}{C_1} \qquad \text{1st Order Increasing} \qquad (5b)$$

If C_0 is the concentration at the beginning of the reaction (zero time), Equations (5a) and 5b) can be written as:

$$k = \frac{1}{t} \log \frac{C_0}{C} \qquad \text{1st Order Decreasing} \qquad (6a)$$

and

$$k = \frac{1}{t} \log \frac{C}{C_0} \qquad \text{1st Order Increasing} \qquad (6b)$$

An expression in the form of Equation (6b) (first order increasing) is often used to determine the bacterial growth rate constant. However, for a process such as exertion of BOD one cannot determine the concentration of BOD at any time, i.e., L_t, unless the initial concentration, L_0, is known, since in the BOD test one determines the amount of BOD expressed, i.e., Y. L_0 can be determined only at the end of the reaction. Also, for the reaeration process, the rate is proportional to the oxygen deficit, i.e.,

$C_s - C_t$. Therefore, one must know the value of C_s, and this, again, can be determined only after completion of the reaction.

The equation for BOD exertion may be written as:

$$\frac{dY}{dt} = K_1(L_0 - Y) \qquad (7)$$

The equation for reaeration may be written as:

$$\frac{dC}{dt} = K_2(C_s - C) \qquad (8)$$

In Equation (7), Y is the concentration of BOD expressed at any time, and K_1 is the velocity constant. In Equation (8), C is the DO concentration at any time, and K_2 is the velocity constant for reaeration. Since the values of L_0 and C_s are not usually known at the beginning of the reaction, but must be determined from experimental data, Equations (7) and (8) are rather difficult to use.

It is in cases such as these that the curve adjustment procedure of Davis can be usefully applied. Since the method was not originally intended for determination of first order reaction constants, it is appropriate to discuss briefly the general principal of the technique before demonstrating the use herein proposed. Davis (2) presented a mathematical treatment of the general semilogarithmic form,

$$y = 10^{a+bx} + \alpha \qquad (9)$$

Figures 1 and 2 show three possible curves for this equation when plotted on semilogarithmic and arithmetic paper. Concerning Equation (9), Davis stated: "Many data that do not show linearity on semilogarithmic paper can be plotted as a straight line by adding to or subtracting from the dependent variable some quantity

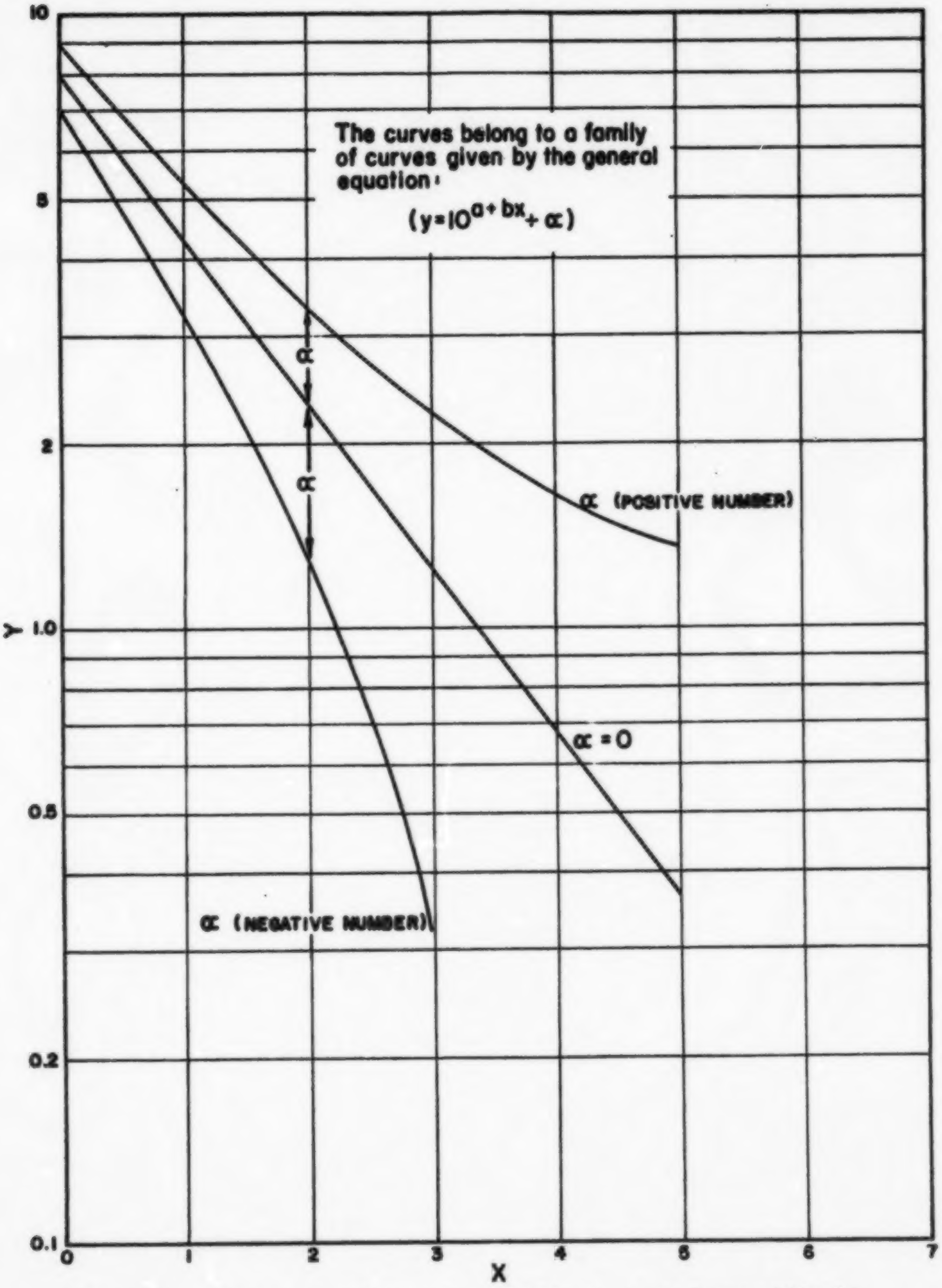

Figure 1 - General plot of modified semi-logarithmic equation.

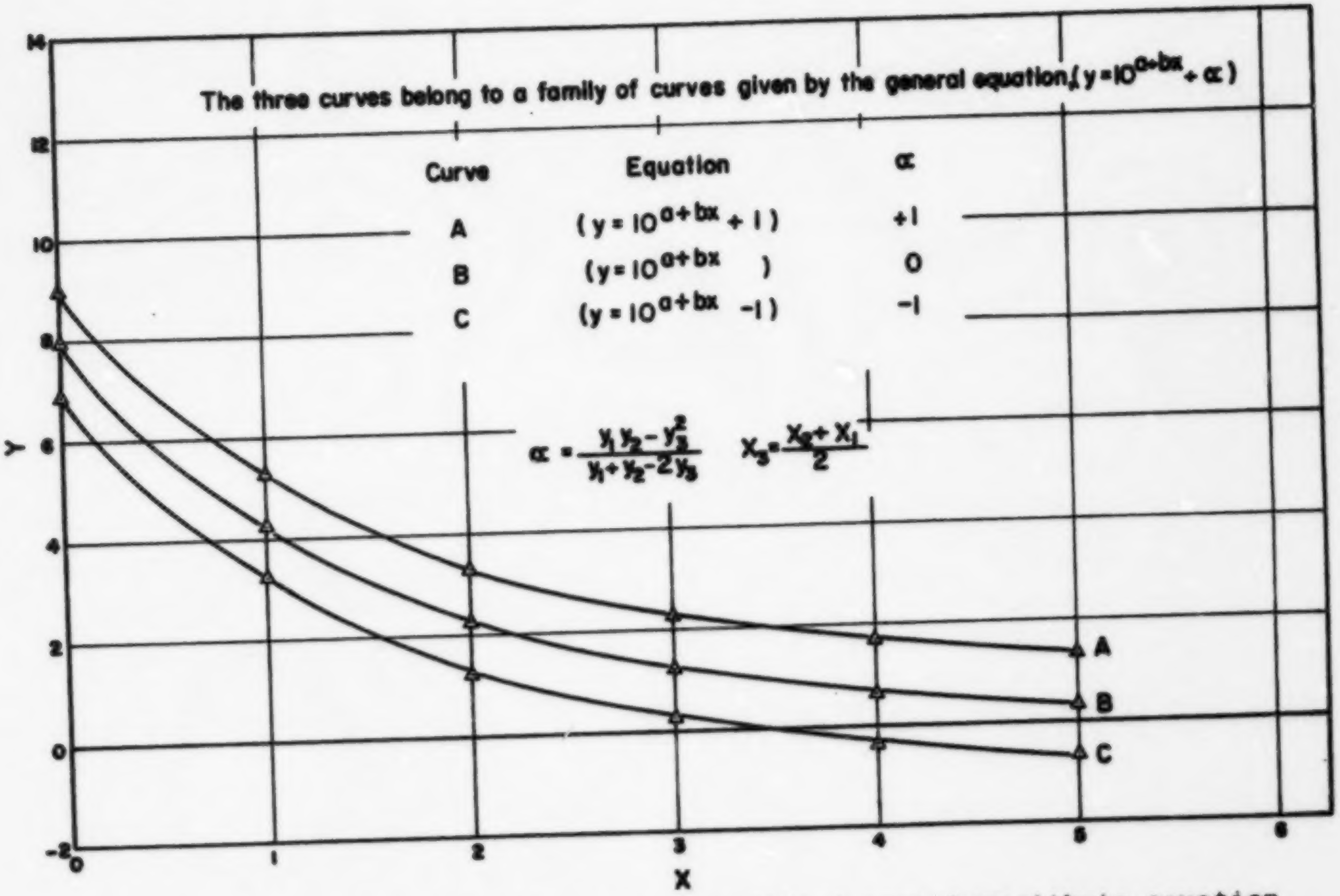

Figure 2 - General arithmetic plot of modified semi-logarithmic equation.

α so that the form of the equation is:

$$y = e^{a+bx} + \alpha \qquad (9a)$$

or, more conveniently

$$y = 10^{a+bx} + \alpha \qquad (9b)$$

In transposing the α term and taking logarithms:

$$\log (y - \alpha) = a + bx \qquad (10a)$$

an indication that plotting $(y - \alpha)$ against x on semilogarithmic paper $\left[\log(y - \alpha) \text{ vs. } x\right]$ leads to a straight line."

Equation (10a) can also be written as

$$\log \frac{(y - \alpha)}{(y_0 - \alpha)} = bx \qquad (10b)$$

Because of the logarithmic scale in Figure 1, it is not immediately apparent that α is a constant. However, Figure 2 shows the same three curves plotted on an arithmetic scale, and it is readily apparent on this figure that the three curves are parallel, hence that α is a constant.

The value of α was treated mathematically by Davis, and was shown to be given by the expression

$$\alpha = \frac{y_1 y_2 - y_3^2}{y_1 + y_2 - 2y_3} \qquad (11)$$

This equation can be derived as follows: A smooth curve is drawn through the points plotted on ordinary coordinate paper and two points (x_1, y_1) and (x_1, y_2) not necessarily data points, are chosen near the extremities of the curve. The arithmetic mean of x_1 and x_2, i.e.,

$$x_3 = \frac{x_1 + x_2}{2} \qquad (12)$$

is computed and y_3 is read as the corresponding x-coordinate (x_3, y_3) from the curve. All points lie on the curve, hence

$$\log (y_1 - \alpha) = a+bx_1$$

$$\log (y_2 - \alpha) = a+bx_2$$

$$\log (y_3 - \alpha) = a+bx_3$$

When these logarithmic equations are solved for x_1, x_2, and x_3, and the values so found are substituted into Equation (12), the following expression results:

$$\frac{\log (y_3-\alpha)-a}{b} = \frac{1}{2}\,\frac{\log (y_1-\alpha)-a}{b} + \frac{\log (y_2-\alpha)-a}{b} \qquad (13)$$

Simplification of this expression and solution for α leads to the previously presented Equation (11)

$$\alpha = \frac{y_1y_2 - y_3^2}{y_1+y_2 - 2y_3}$$

Davis introduced Equation (9), i.e.,

$$y = 10^{a+bx} + \alpha$$

as a purely mathematical approach for use in development of empirical equations defined by data for which the dependent variable could be corrected by a constant α to yield a straight line on a semilogarithmic plot. However, the constant α can be given physical significance and employed as a tool in interpretation of many data that are known or assumed to obey first order kinetics.

APPLICATIONS

Stream Reaeration

In order to illustrate the use of this semilogarithmic form for solution of first order reaction kinetics, the following example of the procedure for analysis is presented:

Recently, the authors undertook a study to investigate atmospheric oxygenation of water as it occurred in a simulated receiving stream (3). Throughout the investigation it was found that DO saturation values reported in Standard Methods (4) were not observed for the tap waters used in the investigation. A method for calculating the _in situ_ DO saturation values for each sample of tap water used was developed and was checked by experimental measurement of actual saturation values.

The Adeney-Becker relation defining oxygen transfer into a turbulent water system is

$$\frac{dD}{dt} = -K_2D \qquad (14)$$

where D is the dissolved oxygen deficit and is equal to the difference between the DO saturation value and the DO concentration value, and K_2 is the reaeration rate constant. This equation can be integrated to yield:

$$\log \frac{D_0}{D_t} = k_2t \qquad (15a)$$

where D_0 equals $(C_s - C_0)$, D_t equals $(C_S - C_t)$, and k_2 is the reaeration rate constant. C_0 and C_t are DO concentration values at time zero and time t, respectively. It can be seen that Equation (15a) is the integrated form of Equation (8).

The saturation value, C_s, must be known in order to accomplish a direct solution of this equation for a time series of observations of DO concentration. Many times accurate values of oxygen solubility are not known for natural waters or for unmonitored atmospheric conditions. In such cases the value of k_2 obtained, assuming applicability of standard published values of DO saturation, may cause errors and inconsistencies in evaluation of this rate constant.

In situ determination of the value of DO saturation can be made from a prolonged time series of observations of DO concentration. Alternatively, Equation (15a) can be solved by assuming some arbitrary value for DO saturation, C_s', and the resulting DO deficits, D_t' can be calculated as $C_s' - C_t$. Equation (15a) can now be rewritten as

$$\log \frac{(D_0' - \alpha)}{(D_t' - \alpha)} = k_2 t \qquad (15b)$$

Equation (15b) is of the same form as the general semilogarithmic equation proposed by Davis, except that Equation (10b) is written for first order increasing rates rather than decreasing rates. The value of error, α, in the assumed D_t' value can be readily determined from an arithmetic plot of C_t versus time; hence, using Equation (11)

$$\alpha = \frac{(C_s' - C_1)(C_s' - C_2) - (C_s' - C_3)^2}{(C_s' - C_1)+(C_s' - C_2)-2(C_s' - C_3)} \qquad (16)$$

or, since D_t' equals $C_s' - C_t$

$$\alpha = \frac{(D_1')(D_2') - (D_3')^2}{D_1' + D_2' - 2D_3'} \qquad (17)$$

D_t' may or may not be the true value of dissolved oxygen deficit; however, so long as the data obey first order kinetics, α can be evaluated and the true DO deficit can be found.

Figure 3 shows an arithmetic plot of the DO concentration versus time for the experimental data given in Table I. Also shown in this figure are the values needed for substitution into Equations (16) or (17) in order to compute α. Once the value of α has been determined, the true value of DO saturation and the reaeration rate constant can be obtained from the following two equations:

$$C_s = C_s' - \alpha \qquad (18)$$

and

$$k_2 = \frac{1}{(t_2 - t_1)} \log \frac{C_s - C_1}{C_s - C_2} \qquad (19a)$$

The experimental data shown in Table I are herein analyzed by the proposed method in order to illustrate its use and to illustrate the effect that DO solubility has upon evaluation of the reaeration rate constant.

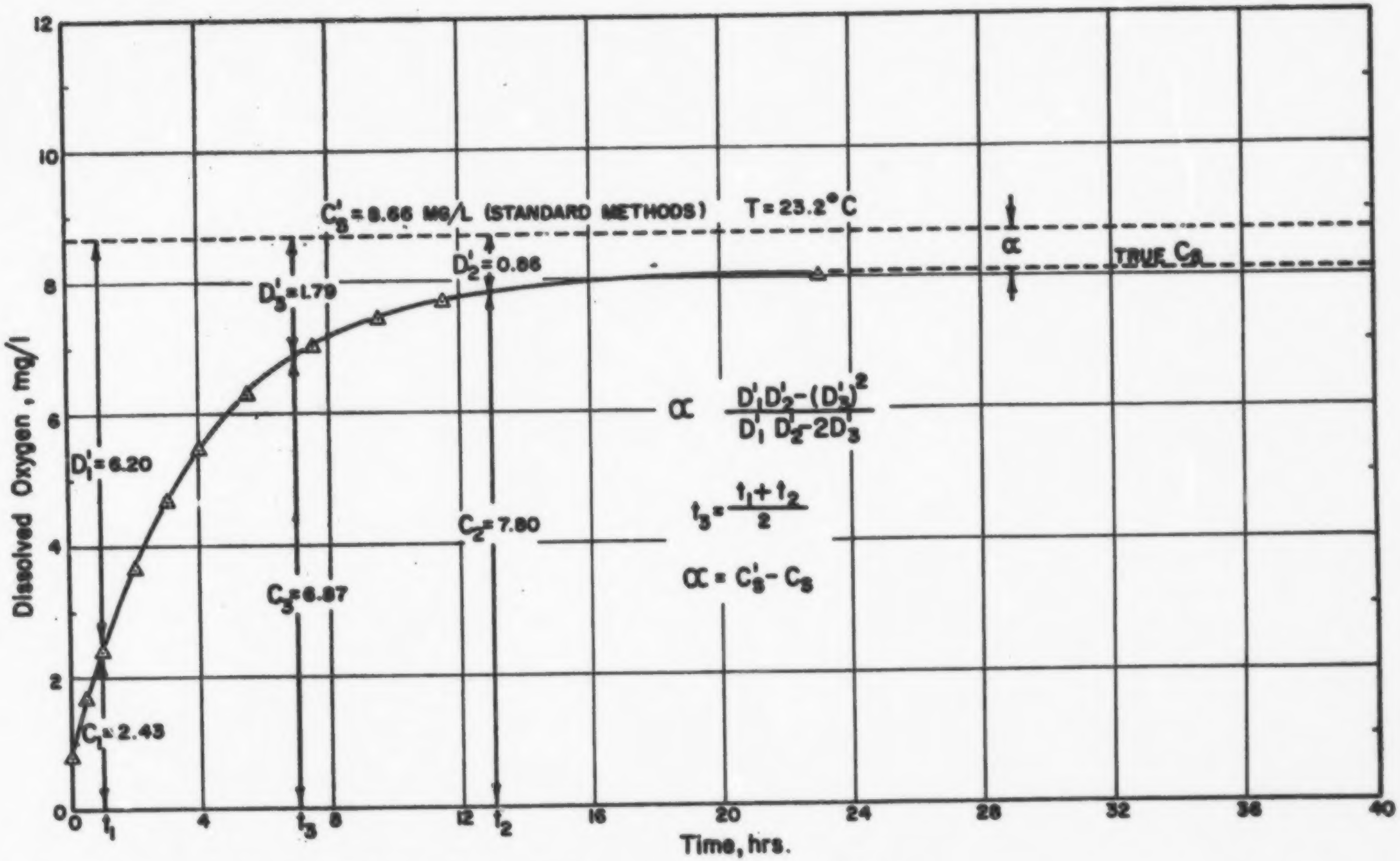

Figure 3 - Determination of α for the reaeration process.

TABLE I

Experimental Reaeration Data

(1) Time (hrs)	(2) Avg. DO Concentration (mg/l) C_t	(3) D' (mg/l)	(4) D(mg/l)
0.0	0.79	7.87	7.26
0.5	1.66	7.00	6.39
1.0	2.43	6.23	5.62
2.0	3.71	4.95	4.34
3.0	4.70	3.96	3.35
4.0	5.49	3.17	2.56
5.5	6.34	2.32	1.71
7.5	7.06	1.60	0.99
9.5	7.47	1.19	0.58
11.5	7.70	0.96	0.35
13.0	7.80	0.86	0.25
23.0	8.05	0.61	0.00

Average temperature during the experiment was 23.2°C. The reported Standard Methods value of C_s is 8.66 mg/l. Values for DO concentration in column 2 are averages of two or more determinations using replicate samples.

The details of the computational procedure are as follows:

(a) Assume $C_s' = 8.66$ (reported Standard Methods value)

(b) Compute D_t' based upon the assumed C_s' value (values computed are shown in column 3, Table I)

(c) Plot C_t versus time on arithmetic paper and select t_1, t_2, and t_3. (Shown in Figure 3, $t_1 = 1$ hr., $t_2 = 13$ hrs., and $t_3 = 7$ hrs.)

(d) Select from the arithmetic plot of C_t versus time, values D_1', D_2', and D_3' (values selected in this example are: $D_1' = 6.20$, $D_2' = 0.86$, and $D_3' = 1.79$)

(e) Compute the value of α

$$\alpha = \frac{D_1'D_2' - (D_3')^2}{D_1'+D_2'-2D_3'} = \frac{(6.20)(0.86) - (1.79)^2}{6.20+0.86-2(1.79)} = 0.61$$

(f) Compute C_s (calculated DO saturation value)

$$C_s = C_s' - \alpha = 8.66-0.61 = 8.05$$

(g) Compute true DO deficit values, D_t, for all observed time periods. (Values are given in column 4, Table I)

$$D_t = D_t' - \alpha$$

(h) The value of k_2 (reaeration constant) can be calculated from Equation (19a).

$$k_2 = \frac{1}{t_2-t_1} \log \left(\frac{C_s-C_1}{C_s-C_2}\right)$$

$$k_2 = \frac{1}{(13.0-1.0)} \log \left[\frac{(8.05-2.43)}{(8.05-0.25)}\right] = 0.113 \text{ hr}^{-1}$$

(i) Least squares analysis for k_2:

Although the value of k_2 can be calculated from Equation (19a) using only two data points, the following least squares analysis using the data for the first 9.5 hours is presented.

The basic equation of semi-logarithmic form is:

$$D_t = 10^{a+k_2 t}$$

Transforming this equation to logarithmic form yields:

$$\log D_t = a+k_2 t$$

The resulting two least squares equations for evalution of k_2 are:

$$\sum_0^n \log D_t = na+k_2 \sum_0^n t \qquad (a)$$

$$\sum_0^n (\log D_t) t = a \sum_0^n t+k_2 \sum_0^n t^2 \qquad (b)$$

The tabulated data for least squares analysis are shown in Table II.

TABLE II

Data for Least Squares Analysis

Time, t	Log D	t Log D	t^2
0	0.86094	0	0
0.5	0.80550	0.40275	0.25
1.0	0.74974	0.74974	1.00
2.0	0.63749	1.27498	4.00
3.0	0.52504	1.57512	9.00
4.0	0.40824	1.63296	16.00
5.5	0.23300	1.28150	30.25
7.5	-0.00436	-0.03270	56.25
9.5	-0.23657	-2.24742	90.25
Totals $\sum_{0}^{n} t$	$\sum_{0}^{n} \log D$	$\sum_{0}^{n} t \log D$	$\sum_{0}^{n} t^2$
33.0	3.97902	4.63693	207.00

Substitution into least square equations (a) and (b) yields:

From Equation (a) $3.97902 = 9a + 33k_2$ (c)

From Equation (b) $4.63693 = 33a + 207k_2$ (d)

Multiplying Equation (d) by 9/33, subtracting from Equation (c) and solving for k_2, gives the value for k_2 of -0.116 hr^{-1}. The above value for k_2 compares very favorably to the value of $k_2 = 0.113\ hr^{-1}$ computed previously (procedure h) using two data points.

Figure 4 shows a plot of D_t and D_t' versus time for the data given in Table I. The curve obtained from the plot of D_t' versus time is displaced from the curve of D_t versus time by the quantity, $\alpha = 0.61$ mg/l. The reaeration rate constant k_2 for the upper curve appears to be continuously changing. Computation of k_2' made for each time period yields the values shown in column 7 on the figure. The values of k_2' were computed by the equation:

$$k_2' = \frac{1}{t_{n+1} - t_n} \log \frac{(D_t')_n}{(D_t')_{n+1}} \qquad (19b)$$

In column 8, k_2' is expressed as percent of the true k_2.

It is common practice in stream survey work to base computations of k_2 on only two observations of C_t, i.e., at the upper and lower ends of the reach of stream under investigation. When one determines the k_2 prevailing between two stations which have different dissolved oxygen levels from one independent sampling period to the next sampling period, computed values of k_2 will vary, regardless of whether the

(1) N	(2) TIME	(3) C_t	(4) D_t	(5) D'_t	(6) $\frac{(D'_t)_n}{(D'_t)_{n+1}}$	(7) k'_2	(8) % OF k_2 $k_2=0.1158$
1	0.0	0.79	7.26	7.87	1.124	0.10154	87.69
2	0.5	1.66	6.39	7.00	1.124	0.10154	87.69
3	1.0	2.43	5.62	6.23	1.259	0.10003	86.38
4	2.0	3.71	4.34	4.95	1.250	0.09691	83.69
5	3.0	4.70	3.35	3.96	1.249	0.09656	83.39
6	4.0	5.49	2.56	3.17	1.366	0.09030	77.98
7	5.5	6.34	1.71	2.32	1.450	0.08069	69.68
8	7.5	7.06	0.99	1.60	1.345	0.06436	55.58
9	9.5	7.47	0.58	1.19	1.240	0.04671	40.34
10	11.5	7.70	0.35	0.96	1.116	0.03177	27.44
11	13.0	7.80	0.25	0.86			

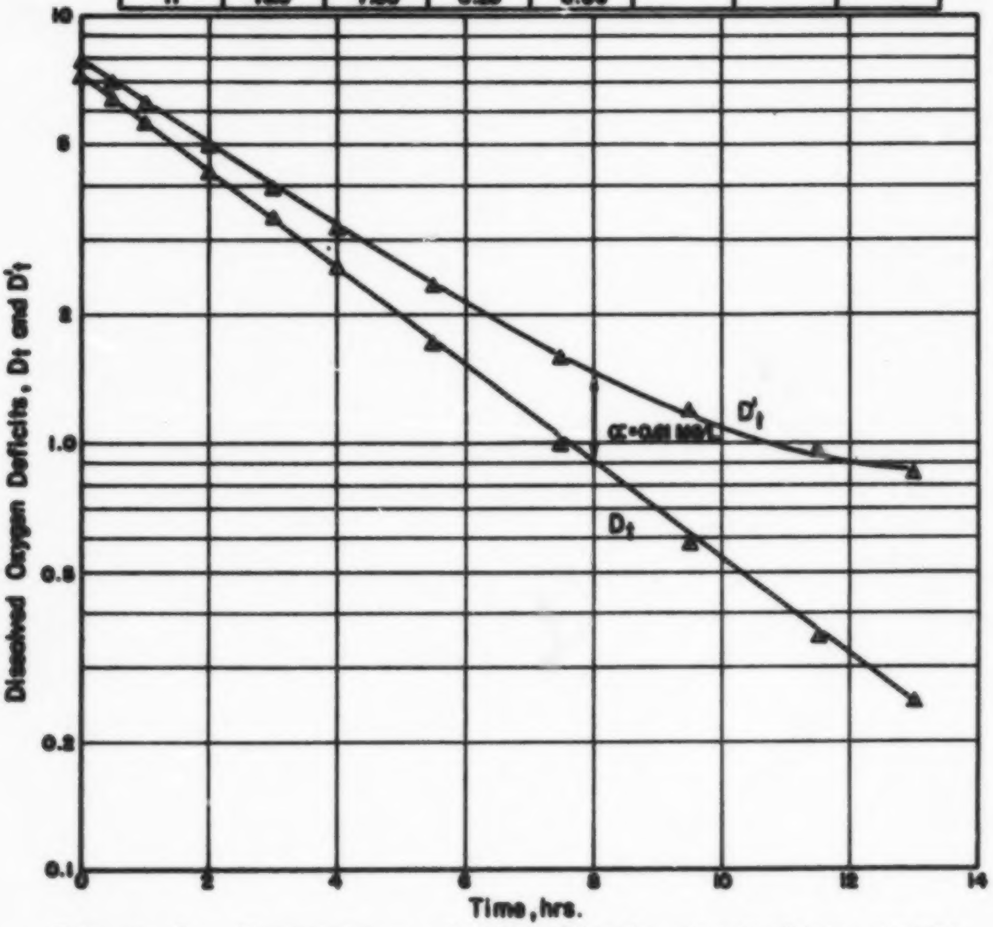

Figure 4 - Comparison of atmospheric reaeration curves based upon the true value for DO saturation and an erroneously assumed value.

hydraulic conditions in the stream remain constant. The values of observed k_2 rates computed in this manner will not remain constant unless the value of C_s' is not in error. It can be seen from Figure 4 that use of the value for C_s taken from Standard Methods led to values of k_2' which varied from 87.69 to only 27.44 per cent of the true value. This magnitude of error indicates that the saturation value used for the water under investigation cannot simply be assumed.

Biochemical Oxygen Demand

The equation for BOD exertion

$$\log \left(\frac{L_0}{L_0 - Y}\right) = k_1 t \tag{20}$$

can be readily solved by application of the general semi-logarithmic equation presented by Davis. Assuming any arbitrary value of L_0' for L_0, and realizing that this value is in error, L_0 can be expressed as:

$$L_0 = L_0' - \alpha \tag{21}$$

Equation (20) can be rewritten as:

$$\log \frac{(L_0' - \alpha)}{(L_0' - \alpha - Y)} = k_1 t \tag{22}$$

Hence, an arithmetic plot of L_t' which is equal to $(L_0' - \alpha - Y)$ versus time enables one to evaluate the error, α, made in assuming L_0' for L_0; therefore

$$\alpha = \frac{(L_0'-Y_1)(L_0'-Y_2)-(L_0'-Y_3)^2}{(L_0'-Y_1)+(L_0'-Y_2)-2(L_0'-Y_3)} \qquad (23)$$

Once α is determined, the ultimate carbonaceous BOD, L_0, is easily obtained from Equation (21). Furthermore, the true BOD remaining at any time can be readily computed from its original definition

$$L_t = L_0-Y_t \qquad (24)$$

Hence, the reaction rate constant can be determined from Equation (20):

$$k_1 = \frac{1}{t} \log \frac{L_0}{L_t}$$

SUMMARY

The authors have utilized a previously proposed mathematical equation (introduced originally for development of empirical equations) as a useful tool for evaluation of first order reaction rate constants. By assigning physical significance to the parameter α, the equation can be utilized in obtaining excellent estimates for limiting boundary values and velocity constants.

ACKNOWLEDGEMENT

This work was supported in part by training grant T1-WP-19, and in part by a research grant WP-00-325 from the Water Pollution Control Administration, Department of the Interior.

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